NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Phytotoxicity Assessment of Water Soluble Fraction of Diesel Using a Marine Microalga: *Tetraselmis chuii* Butcher

Akhere M. A.*& Eze C. O.
Department of Plant Biology and Biotechnology, University of Benin, Benin City 3002 Nigeria
*Corresponding author: mabel.akhere@uniben.edu

Article Info

***Keywords*:**
Tetraselmis chuii, Toxiciy, Diesel, Water soluble fraction, Pollution

Received 6 May 2023
Revised 26 May 2023
Accepted 27 May 2023
Available online 08 June 2023
https://doi.org/10.5281/zenodo.8017325

Abstract

A study was carried out on the effect of the water-soluble fraction of diesel on the growth of a marine microalga, Tetraselmis chui, using appropriate standard methods. Samples were analyzed before and after the experiment for physicochemical parameters such as temperature, total dissolved solids, pH and conductivity. The results showed that different concentrations of water soluble fraction of diesel affected the growth of the microalga. A comparison of the growth responses of the different treatments showed a significant difference of ($p<0.05$) with growth stimulated at lower concentrations of, 0% and 10% of the treatment while at higher concentrations, 20%, 40%, 60%, 80% and 100%, growth was inhibited. There was a significant increase in total dissolved solids, conductivity and pH, while there was a decrease in temperature.

1.0.Introduction

Pollution is an anthropogenic process which causes deleterious effects to organisms or humans [1]. Pollution is of different types which includes land pollution, air pollution or water (freshwater or marine) pollution.

Marine pollution is the introduction of substances into the marine environment which has deleterious effects that cause harm to living resources, hazards to human health, hindrance to marine activities like fishing, diminishing quality of sea water and reduction of amenities [1]. Marine environment is exposed to contaminants of different forms which tend to change the physical, chemical and biological characteristics of the oceans. This contamination can occur as a result of direct discharge of effluents and solid wastes from land or human activities into the sea like run-offs mainly from rivers, atmosphere and shipping which can result in oil spills. The marine environment can serve as a sink for these contaminants which affect the quality of the marine ecosystem and are a potential threat to marine organisms and biodiversity.

Oil fractions are among the major causes of marine pollution [2]. When crude oil or its fractions are present in the ocean, some of its constituents especially the hydrocarbons may dissolve in water and these dissolved components when in high concentrations may become toxic to the marine ecosystem [3].

Petroleum hydrocarbons are complex substances formed from hydrogen and carbon molecules and sometimes contain other impurities such as oxygen, sulphur and nitrogen [4]. They are composed of oil and products refined from oil which include gasoline and diesel. Diesel is one among the most commonly used petroleum hydrocarbon; it is usually used as fuel for ships and it is any liquid fuel used in diesel engines. The most common type of diesel fuel is a specific fractional distillate of

petroleum fuel oil and it can be refined traditionally from petroleum and other materials. When it is refined from oily plants such as soybean and oil palm, it is then referred to as biodiesel.

Water soluble fraction (WSF) also known as water associated fraction is the solution of low molecular mass hydrocarbon naturally released when petroleum hydrocarbon mixtures come in contact with water. It can simply be defined as the fraction of the hydrocarbon that can dissolve in water. Many petroleum hydrocarbons are soluble in water to a limited extent and often contain less soluble, higher molecular mass components and more soluble products of chemical and biological degradation. These components are often harmful to the marine environment. Thus, proper preventive and control measures should be adopted. One of such measures is bioremediation.

Bioremediation is the process whereby biological organisms convert contaminants present in water, land or air into an energy source. It allows the use of natural processes to clean up harmful chemicals in the environment [5]. These biological organisms include yeast, bacteria, fungi, algae and some species of plants. They breakdown harmful substances in petroleum hydrocarbons into less toxic or non-toxic substances by using up the hydrocarbons and organic compounds present in it. Bioremediation is a promising technology and is now being adopted for treatment of polluted environments since it is less expensive than other methods of remediation. A common example of bioremediation is seen in the natural clean-up of oil spills using any of the aforementioned biological organisms. When algae are used as the biological agent, it is referred to as phycoremediaton.

Phycoremediation is an aspect of bioremediation that deals with the treatment of soil and water using algae as the biological organism driving the process. It refers to waste water treatment using algae. It is really useful in the case of wide spread heavy organic and metallic pollution. The algae can use up the contaminant by absorbing it, introducing it into its metabolic processes and converting it enzymatically into harmless substances. Phycoremediation often involves the manipulation of some environmental factors to enhance the process of growth and degradation. Phycoremediation has a lot of benefits which includes the fact that it can be used in the treatment of a wide variety of effluents but can also be highly specific. It is also environmentally safe because it is a naturally occurring process which restores the environment to its initial state after the process has been successfully completed.

Algae as biological organisms have special features that make them suitable for use in phycoremediation. They are chlorophyll containing photosynthetic organisms without vascular tissues but have primitive reproductive structures [6]. They range from unicellular to multicellular forms. Based on size, algae can be classified as microalgae which could either be found in fresh water habitats (*Chlorella vulgaris*) or marine habitats (*Tetraselmis chui*) and macroalgae also known as seaweeds. They act as primary producers both on land and water. They represent a significant biotic component of aquatic ecosystems as they are able to fix carbon dioxide by means of photosynthesis and remove excess nutrients effectively at a minimum cost. Algae species are relatively easy to grow and manipulate in the laboratory and are seen as ideal organisms for use in remediation studies [7]. It has a lot of morphological features which can be influenced by the composition and concentration of various contaminants present in its environment. This objective of this study is to investigate the effect of various water soluble fraction of diesel on the growth of the microalga, *Tetraselmis chui*.

2.0 Methodology

2.1 Source of test microalgae: The marine microalga used was *Tetraselmis chui* and it was imported from Suncoast Marine Aquaculture, located in Florida, United States of America.

2.2 Culture media: The medium used was F/2 medium. The constituent of the F/2 medium was defined by [8], [9]. The medium is suitable for marine algae as it has enriched seawater medium for growing marine algae.

2.3 Culture vessels: 500ml conical flasks were used as the culture vessels for the experiment. The flasks were thoroughly washed with soap, rinsed with water and were later acid washed using

1:1M solution of sulphuric acid (H_2S0_4) to remove contaminants or micro-organisms and thereafter, rinsed thoroughly with water. The laboratory work table was sterilized by swiping carefully with acetone before the culture bottles were placed on the work table. The bottles were immediately closed with cotton wool to prevent contamination by micro-organism(s).

2.4 Preparation of water soluble fraction of diesel: Water soluble fraction of diesel was prepared using the procedure of [10]. The ratio of the mixture was 1:3. 500ml of diesel was added to 1500ml of water in a volumetric flask. The volumetric flask with the mixture was then placed on the magnetic stirrer where it was allowed to stir for 24 hours at room temperature to enable proper mixing. After 24 hours, the mixture was poured in a separating funnel and allowed to stand for 3 hours to obtain a clear interphase between oil and water. Thereafter, a pure and clear water-soluble fraction of diesel obtained at the lower part of the funnel was then placed in capped bottles to make the stock solution used in the preparation the culture media for the experiment.

2.4 Innoculation: To each of the culture bottles, 10ml of each alga was taken with a syringe and inoculated into the culture bottles of concentrations 0%, 10%, 20%, 40%, 60%, 80% and 100% respectively. The bottles were covered with cotton wool to prevent any kind of contamination.

2.5 Growth measurement and monitoring: After inoculation, the growth was measured using a spectrophotometer at 750nm. The spectrophotometer was always zeroed using distilled water. After zeroing, readings were taken by pouring a portion of the concentration into the curvette and the readings were taken for all concentrations of the WSF (0% - 100%). The curvette was rinsed with distilled water after the readings for each concentration was taken. After each day's reading was completed, culture bottles were arranged outside the laboratory windows to ensure exposure of the test algae to adequate light intensity.

2.6 Physicochemical parameters analyses

The samples in the culture vessels were analyzed before and after the experiment for the following parameters: pH, conductivity, total dissolved solids and temperature.

2.7 Analysis of data

2.7.1 Dry Weight: The dry weights were estimated every two days using the formula described by [11] as follows;

Dry Weight = 3.31 + 179.45 × Absorbance at 750nm + 617.45 + (Absorbance at 750nm)^2

2.7.2 Percentage inhibition: Percentage inhibition was estimated at the end of the experiment on day 14 using the formula described by [12] as follows;

$$\textbf{Percentage Inhibition} = 100 - \frac{\text{MeasuredBiomass}}{\text{TheoreticalBiomass}} \times \frac{100}{1}$$

Where Measured biomass = Absorbance of test microalgae in other treatment.

Theoretical biomass= Absorbance of test microalgae in control

2.8 Statistical analysis: Descriptive statistics, inferential (one-way analysis of variance (ANOVA)) and Duncan Multiple Range Test were done using Microsoft excel, 2010 and statistical package for social science SPSS 22.

3.0 Results and Discussion

Figure 1 shows the effect of different concentrations of water-soluble fractions of diesel on *Tetraselmis chui*. Statistically, One-Way ANOVA revealed that there was significant difference ($p<0.05$). Duncan Multiple Range test showed that 0 % and 10 % treatments were significantly different from the other treatments.

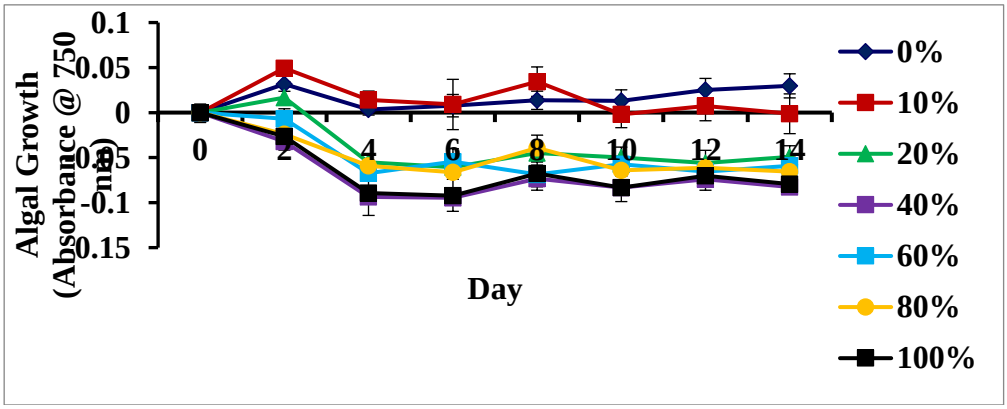

Figure 1: Effect of different concentrations of Water-Soluble Fractions (WSF) of Diesel on *Tetraselmis chui*

The effect of diesel on the growth of *Tetraselmis chui* was studied in the laboratory for 14 days. The results showed that the growth of *Tetraselmis chui* was affected by the water-soluble fraction of diesel.The microalga's growth was stimulated at low concentrations and inhibited at high concentration because of high level of aromatic hydrocarbons. The degree of the toxicity was dependent on the concentration of the water-soluble fraction of diesel used, the level of aromatic hydrocarbons present and the microalgae used. The stimulation of *Tetraselmis chui* growth at low concentration as observed in this study is comparable to the works done by [13], [14] and [15]. However, the growth inhibition that occurred at higher concentrations especially 40% and 100% treatments is in consonance with that reported by [12], [16], [17], [18] and [19]. This can be explained by the fact that diesel oil can interfere with any of the various stages of microalgae's respiration processes like gas diffusion and light penetration due to the coating of the organism [20], glycolysis and oxidative phosphorylation. Diesel oil also contains high levels of aromatic hydrocarbons which explain why its toxicity level is also high [21]. The toxicity of diesel oil leads to cell membrane damage [22] which brings about a reduction in algal growth and [23]also stated that inhibition of cell division processes can also bring about a reduction in algal growth.

Figure 2 shows the percentage inhibition effect of different concentrations of the water-soluble fraction of diesel on the growth of *Tetraselmis chui*. 40% treatment had the highest inhibitive effect on the growth of *Tetraselmis chui*. This was closely followed by 100% treatment. The treatment had the least inhibitive effect.

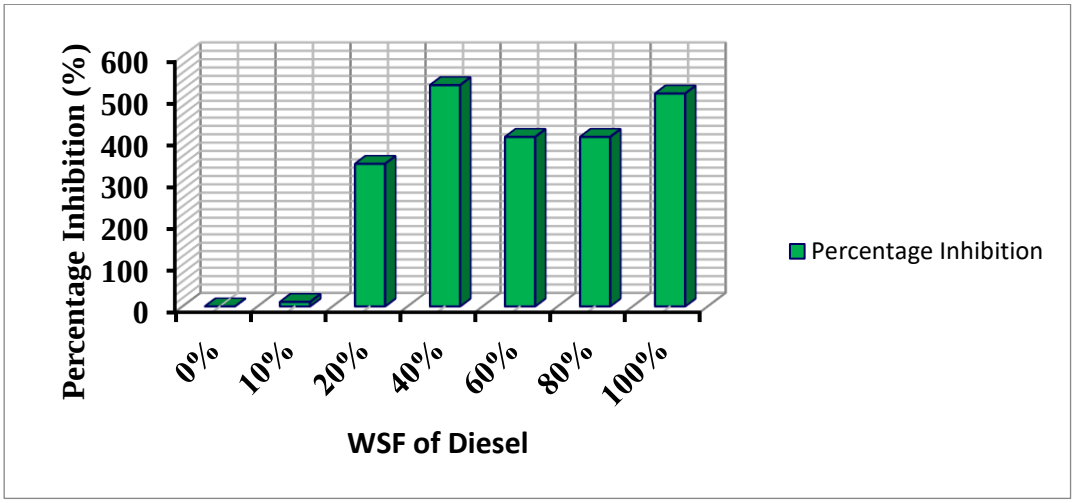

Figure 2: Inhibitive Effect of different concentrations of Water-Soluble Fraction (WSF) of Diesel on *Tetraselmis chui*

Figure 3 shows the pH at the beginning and end of the experiment. All treatments had stable pH at the start of the experiment. pH values increased through days 2, 4, 6 and 8 and declined on day 10. It further increased on days 12 and 14.

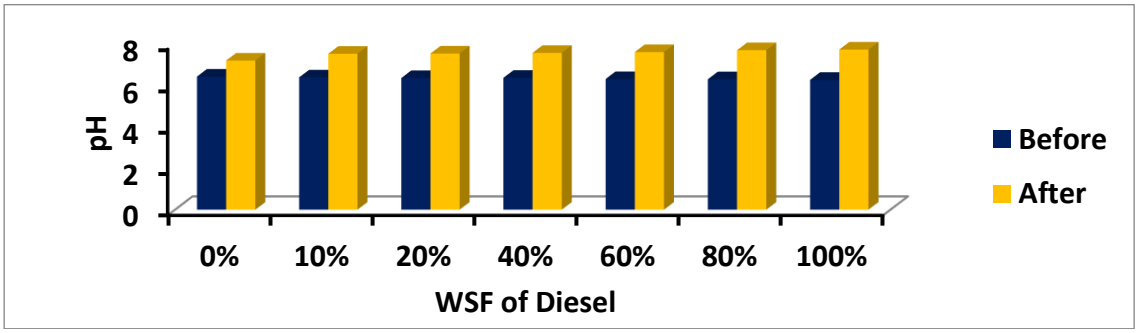

Figure 3. pH before and after the period of study

The pH value was studied in this experiment. pH is a property that can affect microalgae growth. The pH at the start of the experiment for all the treatments were stable and around 6.4 but increased at the end of the experiment with the 100% treatment having the highest pH. This result is not in consonance with the study reported by [24] and [25]. The study by [7] showed there was a decline in growth of the marine phytoplankton *Dunaliella tertiolecta* cultured in wastewater at pH values approaching 9 even with excess nutrient presentand [25] also in their study on *Thallasiosira pseudonana* observed a decline in the alga's growth at pH value of 8.8.

Figure 4 shows the temperature at the beginning and end of study. At the beginning of the experiment, the temperature was high and around 26.4^{o}C – 26.5^{o}C but at the end of the experiment, the temperature greatly reduced with its values around 24.8^{o}C – 25.2^{o}C. It was observed that treatment 0% had the highest temperature and treatments 20 to 100% were stable. Treatment 100% had the lowest temperature on day 6.

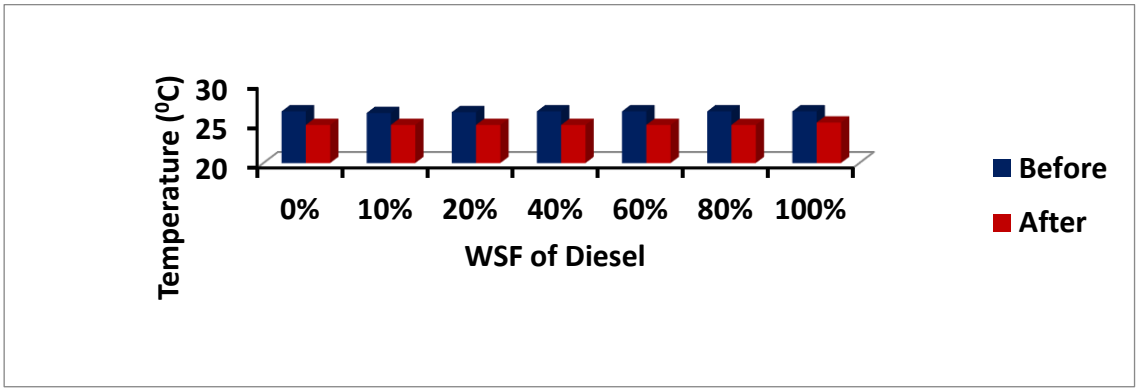

Figure 4: Temperature before and after the period of study

Temperature was also studied in the experiment. This is another sensitive factor for algal growth and metabolic processes. The temperature before the beginning of the experiment was high and around 26.4 to 26.5^{o}C but at the end of the experiment, it greatly reduced and this is a reason for the alga's growth inhibition. This has been reported in different studies like that of [26] who investigated the effect of temperature on the growth of the diatom *Chaeteceros calcitrans* and observed that the algae performed its best at the temperature range of 20^{O}C -30^{o}C and [12] also observed in their

study that at optimum temperature, the cell density of *Chaeteceros calcitrans* was significantly higher meanwhile at lower temperature, the cell density of the diatom was reduced. Another study carried out by [27] also illustrated induced cell proliferation due to increased growth temperature and reported that it was probably due to the changes of the cell's metabolic activities in response to environmental stress.

Figure 5 shows the TDS during the period of study. It was observed that treatments 10%, 20%, 40%, 60%, 80% and 100% had the highest TDS on day 8 while treatment 100% had the lowest TDS on day 0 and 2.

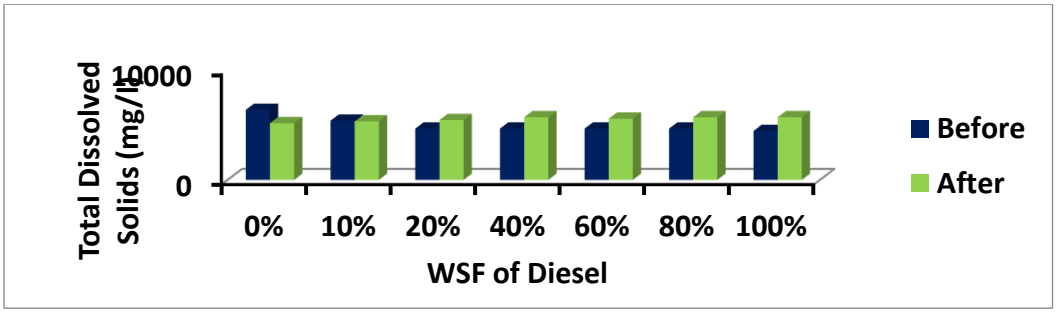

Figure 5: Total Dissolved Solids before and after the period of study

The TDS values at the beginning of the experiment was high at 0% and least at 100% but at the end of the experiment, all the TDS values increased with the 100% treatment having the highest TDS value while the 0% treatment had the least TDS value.

This is comparable to the studies carried done by [28] and [29] that suggested that an increase in TDS can lead to an increase in chlorophyll a content, more nutrient availability and increased productivity.

Figure 6 shows the conductivity at the start and end of the period of study. It was observed that treatments 10%, 20%, 40%, 60%, 80% and 100% had the highest conductivity on day 8 while treatment 100% had the lowest conductivity on day 0 and 2.

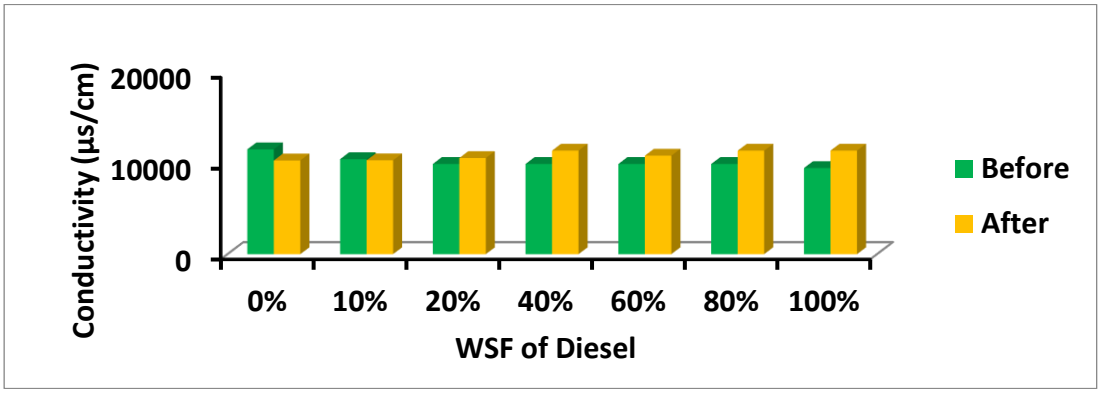

Figure 6: Conductivity before and after the period of study

The Total Dissolved solids (TDS) and Conductivity values at the beginning of the experiment was high at 0% treatment and least at 100% but at the end of the experiment, the TDS and conductivity values increased and the 100% treatment had the highest TDS and Conductivity value while the 0% treatment had the least TDS and Conductivity values. It was reported by [30] that concentrations with high TDS leads to damage of the organism's cell, reduced photosynthetic activity and an

increase in the water temperature. This report can also be used to also explain the effect of high Conductivity levels which is seen as the total TDS within the solution.

4.0. Conclusion

The coastal water is vulnerable to diesel oil pollution due to anthropogenic activities, yet it does not inhibit the growth of marine microalgae, unless there is massive spillage The findings of this study showed that the growth of *Tetraselmis chui* was affected by water-soluble fraction of diesel by inhibiting its growth at high concentrations and stimulating its growth at low concentrations. Since growth is stimulated at low concentrations of WSF of diesel, T*etraselmis chui* can be used in phycoremediation of diesel oil polluted marine environment at low levels of pollution. Further studies using other microalgae species and WSF of diesel should be carried out to ascertain microalgae species that can be used as bioindicators of diesel oil polluted marine environment.

References

[1] R. Beiras (2018). Marine Pollution: Sources, Fate and Effects of Pollutants in Coastal Ecosystems Elsevier 408p.
[2] A. Carpenter. (2019). Oil pollution in the North Sea: the impact of governance measures on oil pollution over several decades. Hydrobiol. Vol. 845(1) pp 109-127.
[3] B. E. Hunnie, L. Schreiber, C. W. Greer and G. A. Stern. (2023). There recalcitrance and potential toxity of polyaromatic hydrocarbons within crude oil residues in beach sediments of the BIOS site, nearly forty years later. Environ. Res. Vol. 222 pp 115329
[4] F. Aminzadeh and S. N. Dasgupta (2013). Geophysics for Petroleum Engineers. Elsevier science. Oxford, UK. 344pp.
[5] G. O. Anoliefo and B. Ikhajiagbe (2012). Techniques in bioremediation. In: Okhuoya, J. A, Okungbowa, F. I and Shittu, H. O (eds). Biological Techniques and Application. Uniben Press, University of Benin, Ekehuan campus, Benin City. Pp 211-212.
[6] F. I. Opute and M.O. Kadiri (2013).Phytoplankton Algae of Nigeria. Mindex Press, Benin city, Nigeria. 304p.
[7] K. Dresback, D. Ghoshal and A. Goyal. (2001). Phytoremediation of trichloroethylene (TCE). Physiol. and Molecular Biol. of Plants Vol.7, pp 117-123.
[8] R. R. L. Guillard and J. H. Ryther (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian J. of Microbiol. Vol. 8, pp 229-239.
[9] R. R. L. Guillard (1975). Culture of phytoplankton for feeding marine invertebrates.*In*: Smith W. L. and Chanley M. H. (eds.).Culture of Marine Invertebrate Animals. Plenum Press, New York, USA. pp. 26-60.
[10] O. Afolabi, S. A, Adeyemi, and A. M. A. Imevbore (1985).Studies on toxicity of Nigerian crude oils to some aquatic organisms. In: Proceedings of International Seminar on Petroleum Industry and the Nigerian Environment, *NNPC*. pp. 269-273.
[11] J. Horvatic, G. Palijan. and J. Lukavsky. (2003). Algal responses to nutrient additions in water of Nature Park KopackRit (Croatia) by miniaturized algal growth bioassay. Algological Studies Vol. 110, pp 117-126.
[12] P. V. Phatarpekar and Z. A. Ansari (2000*)*. Comparative toxicity of water soluble fraction of four oils on the growth of a microalga. Botanica Marina Vol. 43(4), pp 367-375.
[13] N. J. Prouse, D. C. Gordon and P. D. Keiser (1976). Effects of low concentration of oil accommodated in seawater and the growth of the unialgal marine phytoplankton cultures. Journal of Fisheries Research Board Canada Vol. 33, pp 810-818.
[14] M. Karydis (1979). Short Term Effects of Hydrocarbon on the Photosynthesis and Respiration of some phytoplankton species. Bot. Mar. Vol. 22, pp 281-285.
[15] D. Bhattacharje and O. J.Fernando (2008).Short term studies on the effect of water soluble fractions of diesel on the growth of *Chaetoceros calcitrans*, Paulsen. Res J. of Environ. Toxicol. Vol. 2 (1), pp17-22.
[16] M. F. Eman and S. M. Mohamed (2006). Impacts of water extract of diesel oil and gasoline fuels on growth and some biochemical activities of *Dunaliella salina* Teod. Egyptian J.of Phycol. Vol. 7(1), pp 17-32.
[17] M. Kadiri and D. Eboigbodin (2012). Phytotoxicity assessment of water soluble fraction of refined petroleum products using microalgae. Acta Bot. Hungarica Vol. 54(3-4), pp 301-311.
[18] S. Yakub. And M. R. Ajijo. (2016). Assessment of acute toxicity of water soluble fraction of diesel on two species of marine microalgae. J. of Aquat. Sci. Vol. 31(2B), pp 383-390.

[19] S. Salinas-Whittaker, C. Gómez-Gutiérrez, B. Cordero-Esquivel, P. A. Luque. G. Guerra-Rivas. (2020). Effects of the water-soluble fraction of mixture of fuel oil /diesel on the microalgae *Dunaniella tertiolecta* through growth. Environ.Sci. and Pollution Res. Vol. 27, pp 35148-35160.
[20] C. S. Lobban and P. J. Harrison (1997). Seaweed Ecology and Physiology. Cambridge University Press, Cambridge, U.K. 66p.
[21] I. Lysyj and E. C. Russel (1974). Dissolution of petroleum derived products in water. Water Res. Vol.8(11) pp. 863-868.
[22] P. B. Kauss and T. E. Hutchinson (1975).The effects of water soluble petroleum components on the growth of *Chlorella vulgaris* Beijerinck, Environ. Pollution.Vol. 9, pp 159-174.
[23] M. Piehler, V. Winkelman, L. Tworney, N. Hall, C. Currin and H. Paerl (2003). Impact of diesel fuel exposure on the microphytobenthic community of an intertidal sand flat. J. Exp. Mar. Biol. Ecology Vol.297, pp 219-237.
[24] J. C. Goldman (1976). Phytoplankton response to waste water nutrient enrichment in continuous culture. Journal exp. Mar. Biol. and Ecology Vol.57:pp 1-13.
[25] G. D. Pruder and E. T. Bolton (1979). The role of CO_2 enrichment of aerating gas in the growth of an estuarine diatom. *Aquaculture.*17: 1 – 15.
[26] S.A. Nurul, M. Y. Fatimah and Mohamed Shariff (2013). Effect of salinity and temperature on the growth of diatoms and Green Algae. Journal of Fisheries and Aquatic Sciences Vol. 8, 397-404.
[27] J. Sheehan. (1998). A look back at the U.S Department of Energy's Aquatic species Program. Biodiesel from Algae. *National Renewable Energy Laboratory, USA*. 325p.
[28] G. Seenayya (1973). Ecological studies in the plankton of certain freshwater ponds of Hyderabad, India. Hydrobiology Vol. 41(4), pp. 529-540.
[29] J. Kerekes and J. R. Nursall (1966). Eutrophication and senescence in a group of Praiie-Parkland lakes in Alberta, Canada. Limnology Vol. 16(1), pp 65-73.
[30] M. K. Mitchell and W. B. Stapp (1992). Field Manual for Water Quality Monitoring, an environmental education program for schools. GREEN