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Research Article

PHYTOTOXICITY AND EFFECT OF WATER SATURATED FRACTIONS OF HYDROCARBONS ON GROWTH AND PHOTOSYNTHETIC PIGMENT OF MICROALGAE

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ABSTRACT

Phytotoxicity and the effects of water saturated fractions of hydrocarbons on growth and photosynthetic pigments of microalgae was carried out 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.

Keywords: Phytotoxicity, water saturated fractions, hydrocarbons, growth, Photosynthetic pigment and microalgae.

INTRODUCTION

The effect of crude oil and petroleum hydrocarbons on micro algae had been investigated for some decades to address the problem of oil pollution. A major part of the research activities related to oil pollution in recent years has centered on acute and chronic effect of crude oil and specific hydrocarbons on algae. Most of the literature available lay emphasis on the crude oil and hydrocarbons themselves and not on the soluble fractions [1-3, 6, 11 and 12]. There is however, scanty literature on the effect of water soluble fractions of crude oil, hydrocarbons and isomers on the algal community. The effects of crude oil pollution on growth and metabolic activities of the fresh water algae *Chlorella homosphaera* and *Chlorella vulgaris* was investigated by [14]. According to the authors, the presence of crude oil or its refined products in the culture media of algae markedly influenced their growth, protein and nucleic acid content. Toxicity of the oil was reported to be concentration dependent. Low concentration stimulated growth, protein content and nucleic acid while higher concentrations had inhibitory effect. DNA and RNA responded similarly, in crude oil DNA exhibited more sensitivity than RNA in the two test organisms. The authors concluded that different species of algae responded differently to oil pollution. The degradation pattern of crude oil by marine cyanobacteria (*Oscillatoriasalina*, *Plectonematereberans* and *Aphanocapsissp*) was investigated by Raghukumar, Vipparity, David and Chandramohan [11]. These cyanobacteria degraded Bombay high crude oil when grown in artificial seawater. Oil removal was measured and around 45-55% of the total fractions of crude oil containing 50 aliphatic, 31 waxes, 17 bitumen, 14% aromatics and 5% polar compounds were removed in the presence of these algae within 10 days. Between 50% and 65% of pure hexadecane and 20% and 96% of aromatic compound (Anthracene and phenanthrene) disappeared within 10 days. Mixed culture of Cyanobacteria species removed over 40% of the crude oil. The authors reported that excellent Cyanobacterial mat was formed in mixed culture and thus the potential for use in mitigating oil pollution on seawater, either individually or in combination. Crude oil is a homogeneous substance, apart from being a homogeneous compounds it comprises different complex mixture of thousands of different chemical compounds. In addition, the composition of each is unique and it varies in different producing regions and even in different unconnected zones [17]. The composition of oil also varies with the amount of carbon atom present. Significantly, the many compounds in oil differ markedly in solubility, and susceptibility to biodegradation. Some compounds are readily degraded; others stubbornly resist degradation; still others are virtually non-biodegradable. The biodegradation of different petroleum compounds occurs simultaneously but at very different rates [21].The main component of the vertical flux of samples taken from two tanks with oil concentrations of 4.5µg/l (low tank) and 13.5µg/l (high tank) in a seven day experiment were reported to be amorphous suspended matter, mostly originating from dead phytoplankton and living diatoms [22]. It was further observed that vertical transport of oil emulsions were probably conducted after absorption to amorphous suspended matter and living diatoms which settled in the sediment trap at the bottom of the tanks. The objective of this study is to examine the phytotoxicity and the effects of water saturated fraction of hydrocarbons on the growth and photosynthetic pigment of fresh

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water microalgae. Patents reviewed show a number of uses for algae. Their uses as staple food in Asia country as and biofuel were reported by [15]. Microalgae fermentation using illumination was reported by [30]. The author noted that high quality eggs with high omega 3- fatty acid content can be produced by poultry by feeding birds with high elevated amount of defatted microalgae. Under this condition poultry are capable of producing egg comprising about 300mg to about 550mg of n-3 fatty acids. The authors reported some of the appeal of algae to include their fast growth rate compare to convectional terrestrial plants and simple nutritional requirement.

MATERIALS AND METHODS

Test Microalgae

Three microalgae were used as experimental microalgae. These were Chlorella vulgaris, Nitzschiapalea, and Anabaena flosaquae[23].

Culture Medium

The microalga species was grown in an artificial batch culture prepared according to Chu's modified No 10 medium [9].

Table 1:Composition of the modified Chu no 10 culture medium

- A stock solution was made by dissolving the salts listed in the amount indicated (in grams) each in 100ml of distilled water.

SALTS/NUTRIENT	g/100ml
$CaCl_2.2H_2O$	3.67
$MgSO_4.7H_2O$)	3.69
$NaHCO_3$	1.26
K_2HPO_4	0.87
$NaNO_3$	8.5
Na_2SiO_3	2.84

- An iron solution was prepared by dissolving 3.35g citric acid ($C_6H_8O_7.H_2O$) in 100ml distilled water, then 3.35g ferric citrate $FeC_6H_5O_3.5H_2O$ was added. This mixture was autoclaved and refrigerated (-0^0C) in darkness by wrapping in aluminum foils.
- A trace element solution was made by dissolving the salts below in the amounts (mg) indicated in 1 L of distilled water. The mixture was autoclaved and kept sterile.

Table 2: **Trace elements composition of the modified Chu no 10 culture medium**

SALT	Mg/l
$CuSO_4.5H_2O$	19.6
$ZnSO_4.7H_2O$	44
$CoCl_2.6H_2O$	20
$MnCl_2.4H_2O$	36
$NaMO_4.2H_2O$	12.6
H_3BO_3	618.4

Table 3: Vitamin stock

VITAMINS	g/100ml
Cyanocobalamin (B_{12})	0.004
Thiamin	0.004
Biotin	0.004

The final culture medium was prepared by adding aseptically, 1ml of each of the six stock solutions in step (a) above to 1ml of stock solution in step (b) above to 1ml of each of the stock solution in step (c) above and 1ml of vitamin stock.

The resulting solution was then made up to 1L with distilled water in 1 litre volumetric flask

Culture Vessels

Nine hundred and fifty ml round bottomed transparent glass bottles were used for both sub culturing and in the main experiment. They were washed thoroughly with detergent and further rinsed with solution of nitric acid and sulphuric acid separately to remove any algal spore present.

Isolation of Pure Culture of Microalgae

Unialgal culture of the microalgae used for this investigation was isolated from a water body collected around Benin metropolis. This was subjected to series of sub culturing in modified Chu 10 artificial growth medium. An aliquot of the water sample collected was taken and used as inoculum to inoculate the growth medium and allowed to grow. Microscopic examination and identification of microalgae was made using relevant texts books and an algal album in the phycology and environmental laboratory of University of Benin. [16 and 24].

Hydrocarbon Source

The hydrocarbons used (Hexane, Xylene, Benzene and Toluene) for this study was purchased from Thomas Gold venture in Benin City Edo state.

Preparation of Water Saturated Fractions (WSF)

The WSF was prepared in 1:9 according to the method of [4 and 24]. A sample of the various hydrocarbons was slowly mixed separately in equal volume of distilled water in ratio 1:9 in a 2L screw-cap conical flask. This was placed on Gallen-kamp table top magnetic stirrer and stirred with 7/cm magnetic rod for 24hrs at room temperature (27^0C ± 2^0C). After mixing, the oil-water mixture was allowed to stand overnight in a separating funnel. The water saturated fraction was separated from the hydrocarbon residue and referred to as stock or 100% WSF. The stock WSF was diluted with the culture medium serially to give 10%, 25%, 50%, 75% and 100% WSF

Experiment Design

From the 100 stock solutions a range of various WSFs were prepared. The concentrations ranged with a lower concentration of 10% followed by 25%, 50%, 75% and maximum of 100% by the addition of 10, 25, 50, and 75 ml of WSF respectively and this is made up to 100ml with the growth medium. A control was maintained in which no WSF was added. All concentrations were run in triplicate.

Inoculation

Four hundred and fifty ml each of the water saturated fractions of the above hydrocarbons were measured separately in the experimental vessels. These were then inoculated separately using 2mls of each of the experimental microalgae with a 5ml syringe. Each experiment was set up in triplicate. The experimental bottles were plug with sizeable cotton wool to limit evaporation and prevent contamination from the environment. The cultures were then place in a southern window for solar illumination. Fifty ml aliquot was taken from each triplicate and compounded into a one hundred and fifty ml composite sample every other day for analysis.

Growth measurement:

Two growth indices were used to evaluate the effect of the various water saturated fractions on the microalgae. They include:

(i) Absorbance (ii) Chlorophyll- *a*

Absorbance: Twenty five ml aliquot were collected every other day. The optical density was then determined at 745nm using HACH DR 2000 Spectrophotometer.

Photosynthetic pigment Chlorophyll– *a, b* and *c*

Twenty five ml aliquot was taken for chlorophyll-*a* extraction. This volume was filtered through filter chamber containing a What man membrane filter paper. The filter paper along with its content was then transferred into twenty five ml of alcohol (95% ethanol) and kept in the dark for 12 hours for extraction of chlorophyll pigments and to avoid photo oxidation of the pigments. The extract was read in a spectrophotometer at wave length of 665, 645 and 630. The concentrations of chlorophyll was then determined by the following formula

Chlorophyll- *a* (mg/L) = 11.64 D_{665} - 2.16 D_{645} - 0.1 D_{630}, Chlorophyll-*b* (mg/L) = 20.7D_{665} - 4.34D_{645} - 4.42D_{630}, chlorophyll- *c* (mg/L) = 55.0D_{665} - 16.3D_{645} - 4.64D_{630} [7 and 15). Where, D_{630}, D_{645} and D_{663} are absorbance at 630nm, 645nm and 665nm respectively.

Growth rate determination

The following formula, as described by [16] was used for calculating the growth rate.

$$\text{Growth rate (K)} = \frac{\ln (N/NO)}{T}$$

Where N = Microalga population at time T
NO =Initial microalga population at time zero
T = Time interval from time zero.

Percentage inhibition

Percentage inhibition on the various microalgae was determined using the formula

$$\% \text{ inhibition} = 100 - \frac{\text{Measured biomass}}{1} \times \frac{100}{\text{Theoretical biomass}}$$

Where: Theoretical biomass = biomass of control experiment [25].

Extraction of Total Petroleum hydrocarbon (TPH) and Polyaromatics hydrocarbons (PAH).

Total petroleum hydrocarbons (TPH) and Polyaromatics hydrocarbons (PAH) were analyzed according to the methods described in [7]

- 100ml of a well-mixed sample was transferred into clean separating funnel, previously washed and rinsed with distilled water.
- 50ml of dichloromethane was then added.
- 1ml of surrogate spike standard was added.
- The funnel was then covered and shaken, first gradually and then vigorous. The funnel was vented at interval during the shaking.
- The set up was then allowed to stand to settle and for the layer to separate. The organic layer was then filtered through What man No. 41 filter packed with 10g of anhydrous Sodium sulphate into a round bottomed flask.
- Sample extract was then concentrated using a rotary evaporator at 60^{0}C to about 1-2ml volume and transferred into a sample vial.
- The same was done for blank using distilled water.
- The sample extract and blank are now ready for TPH analysis and for cleanup/ fractionation to get the Aliphatic and Aromatics (PAH).

FRACTIONATION PROCEDURE

Preparation of the fractionation column

- A glass wool fiber was inserted into the column.
- 10g of silica gel (60-200 mesh size, Davidson Grade 850) was weighed. Preconditioned by baking overnight at 105^{0}C and was used to pack the column.
- The base of the column was tapered to pack the silica gel properly.
- The column was then eluted with n-hexane while preventing the column from drying out.

Fractionation of the sample extract

- 1ml of sample extract was transferred to the top of the column using a pipette.
- The column was eluted with 60ml of n-hexane to get the Aliphatic Hydrocarbons. The eluate was then collected in a conical flask.
- While the hexane was almost getting dry, 40ml of dichloromethane was added to elute the PAH components. This was also collected in another conical flask.
- The sample elutes was concentrated to 1-3ml using the rotary evaporator and was transferred into sample vials.
- The same was done for blanks, using n- hexane and dichloromethane, respectively.

Gas Chromatographic Analysis

In the GC analysis, 1µL of the sample extract of the WSF of crude oil sample was injected into the GC column with a sharp plug. The GC column was coated with stationary phase. The column was contained within an oven. The sample was then vaporized to dryness at degree and at a rate dependent on the boiling point. It was then carried by an inert gas (helium) through the column to a detector. The detector then recorder the response in the shape of a Gaussian peak. Several factors determine the rate at which an injected compound travels through the column and reaches the detector. A compound's volatility at the column temperature influences the distribution of the compound between the gaseous mobile phase and the stationary phase. Other things being equal, a compound's band will move through the column more quickly if its distribution favors the mobile phase. Boiling points are often used as a measure of the relative volatilities of compounds in a mixture. However, volatility alone does not determine the distribution between the stationary and mobile phase. Specific interactions between the injected compound and the stationary phase play an important role as well. Recall that "like dissolves like" so a polar compound will tend to strongly distribute into a polar stationary phase. Conversely, a polar compound will not have a strong affinity for a non-polar stationary phase and, thus, will elute relatively quickly. In summary, components in a mixture can be identified by analyzing the difference in their retention times, which is dependent upon their volatility and polarity. The result was then calculated as follows

Sample (mg/L) = (Reading X Dil.F X F. vol) / In. vol (ml)
Where, F.vol = the final volume of the concentrated extract (in ml)
In.vol = the initial volume of the homogenized sample (in ml)
Dil.F = the dilution factor used
Reading = the chromatogram value

Percentage hydrocarbon degredation

This was determined by soxhlet technique as described [7]. Result is being discussed in another article another journal.

Statistical Analysis

Apart from the calculated means, Standard error of mean, Analysis of variance was also calculated to detect significant difference between the levels of toxic effect of various concentrations of the treatment on the different microalgae. Where there was significant difference, the Duncan multiple tests was carried out. The statistical package used was the SPSS version 15.

RESULTS

The responses of *Chlorella vulgaris, Nitzschiapalea*and *Anabaena flosaquae*in different concentrations of water saturated fractions of hydrocarbonsl (WSF) at are shown in fig 1-12. Figures 1-4 show the effect of WSF of hydrocarbons (Xylene, Toluene, Hexane and Benzene) on growth of *Chlorella vulgaris*. The microalga show similar growth responses in the various WSFs. An initial lag growth phase was observed at the early age of the culture between day 0 and 4 in the WSFs except in control and in 10% concentration of WSF of Xylene. Growth stimulation was recorded in lower concentrations (10% and 25%) and mid concentration (50%) between days 4 and 14 while higher concentrations (75% and 100%) were completely inhibitory to the growth of the alga. There was however some exceptions, In the WSF of hexane (fig 3), growth stimulation was recorded in 0% to 75% concentrations and growth inhibition in 100% concentration while in the WSF of Toluene (fig 2), growth stimulation of the alga was observed at lower and mid concentrations while complete growth inhibition was recorded at higher concentrations. Total algal growth synthesized in control was lower than that in low and mid concentrations of WSFs of the hydrocarbons and higher than that in higher concentrations. No statistical difference at ($p< 0.05$) was observed in the growth of the alga in the various treatments.

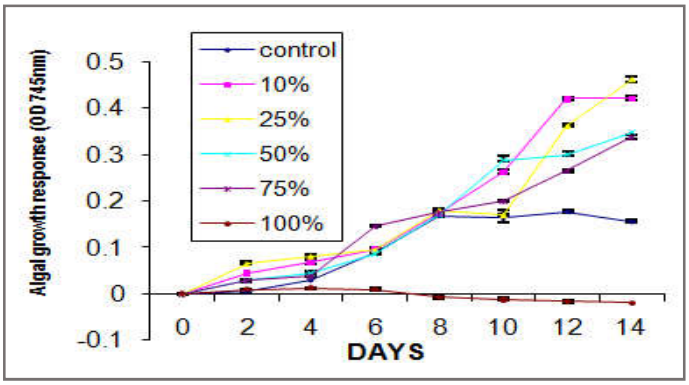

Fig 1: Response of *Chlorella vulgaris* in different concentrations of WSF of Toluene

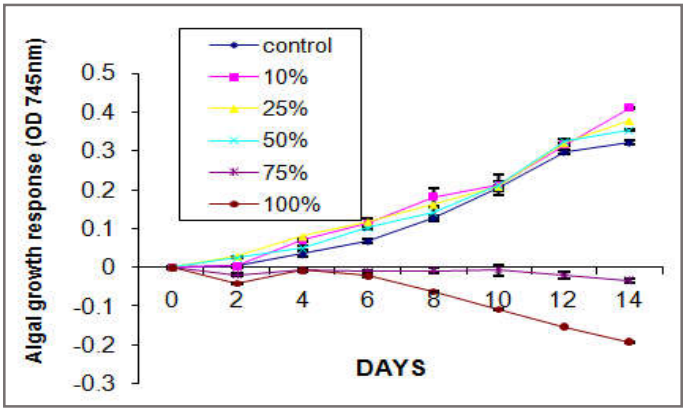

Fig 2: Response of Chlorella vulgaris in different concentrations of WSF of Xylene

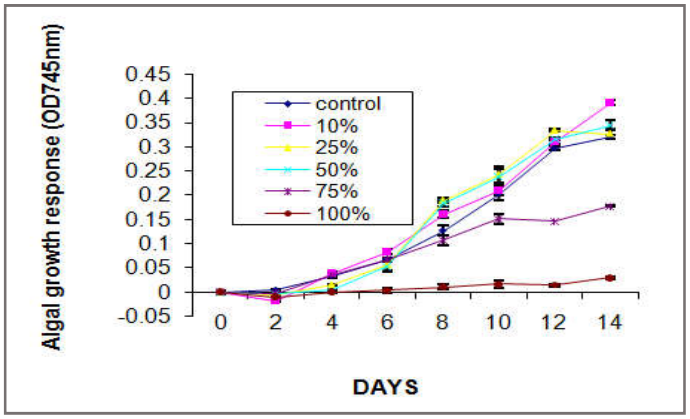

Fig 3: Response of Chlorella vulgaris in different concentrations of WSF of Hexane

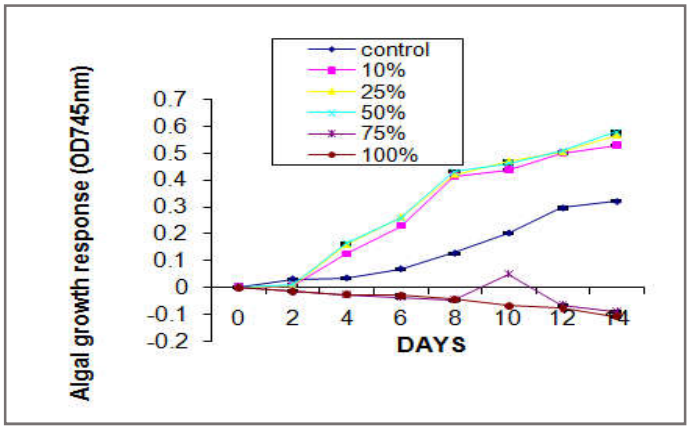

Fig 4: Response of Chlorella vulgaris in different concentrations of WSF of Benzene

The growth responses of *A. flosaquae*in different concentrations of WSF hydrocarbons (Xylene, Toluene, Hexane and Benzene) are shown in fig 5-8. The microalga showed growth stimulation in 0% - 50% concentrations in the WSFs of Toluene (fig 6), 0% - 75% in Xylene (fig 5) and Benzene and 0% - 100% in Hexane (fig 7) after an initial lag phase of growth between day 0 and 2 in higher concentrations. Algal growth inhibition was only observed in higher concentrations except in the WSF of hexane where there was no inhibition of growth at all concentrations. Total algal growth in the control was higher than that in all investigated concentrations and there was no statistical difference at ($p< 0.05$) was observed in the growth of the alga in the various treatments.

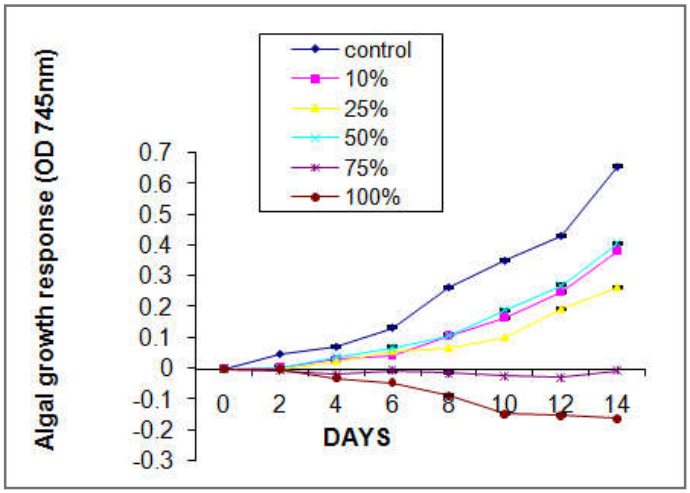

Fig 5: Response of Anabaena flosaquae in different concentrations of WSF of Toluene

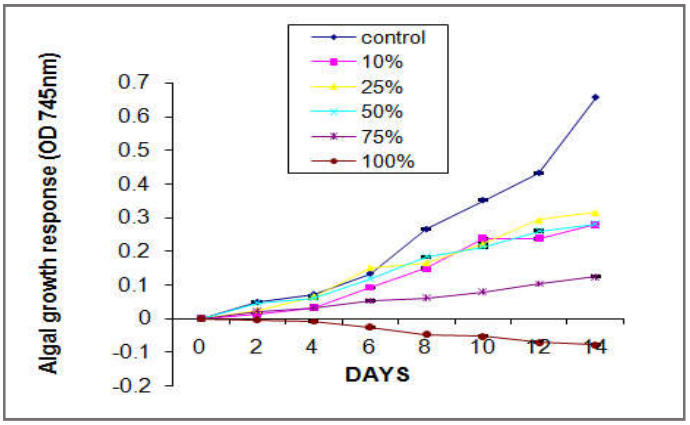

Fig 6: Response of Anabaena flosaquae in different concentrations of WSF of Xylene

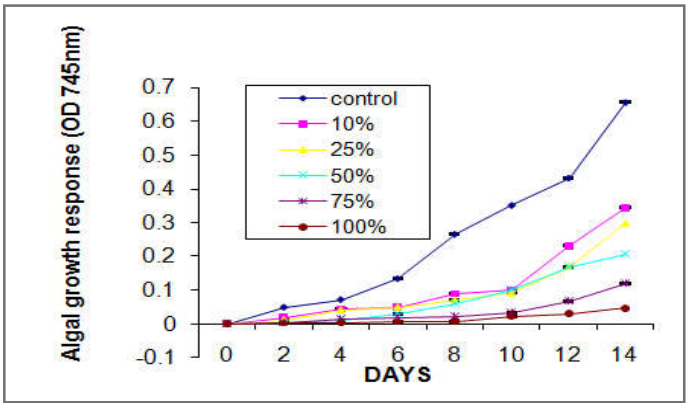

Fig 7: Response of *Anabaena flosaquae* in different concentrations of WSF of Benzene.

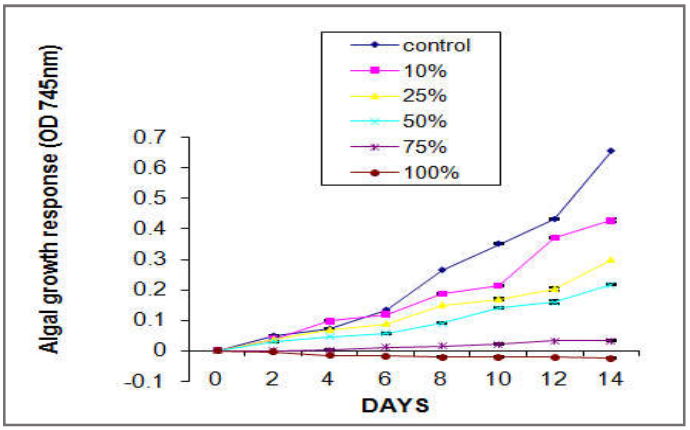

Fig 8: Response of *Anabaena flosaquae* in different concentrations of WSF of Hexane

Figures 9-12 show the effect of WSF of hydrocarbons (Xylene, Toluene, Hexane and Benzene) on growth of *Nitzschiapalea*. The figures show remarkable and exponential growth stimulation at all concentrations of the various WSFs investigated. In almost all cases, there was no lag phase of growth and growth stimulation was spontaneous from day 0 to day 14 of the experiment. Total algal growth in treatment cultures was higher than that in the control experiment.

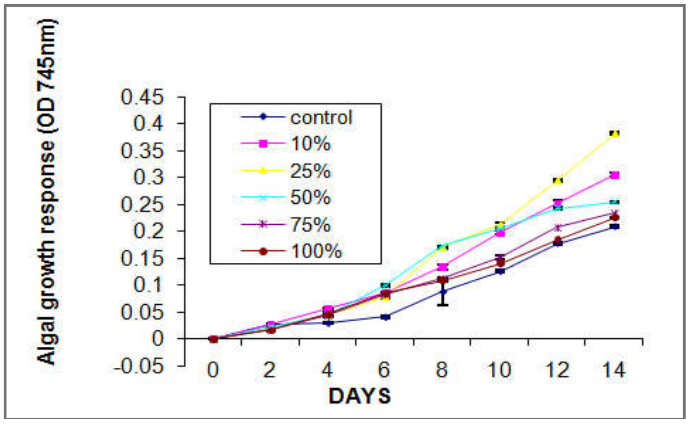

Fig 9: Response of Nitzschiapalea in different concentrations of WSF Xylene

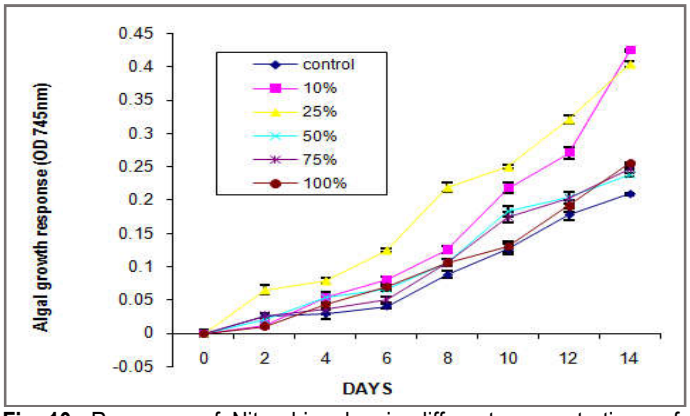

Fig 10: Response of Nitzschiapalea in different concentrations of WSF Toluene

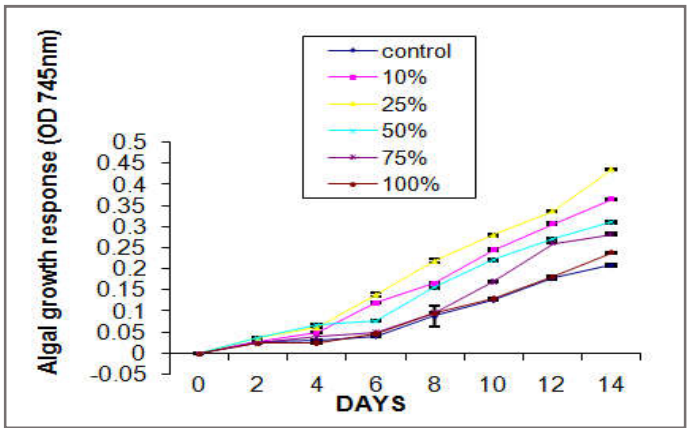

Fig 11: Response of Nitzschiapalea in different concentrations of WSF of Hexane

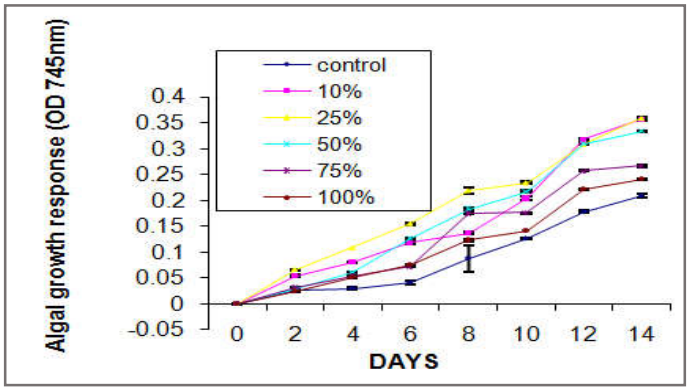

Fig 12: Response of *Nitzschiapalea* in concentrations of WSF Benzene

Table 1: Cumulative photosynthetic Pigment in WSF of hydrocarbons across concentration gradient

Microalgae/Days		*Anabaena flosaquae*	*Nitzschia palae*	*Chlorella vulgaris*
Xlyene	DAY 0	0.116	4.34	4.86
	7	0.120	2.92	2.64
	14	0.140	3.21	3.05
	SEM	± 0.150	± 0.512	± 0.459
Benzene	DAY 0	0.116	4.34	4.86
	7	0.104	1.67	1.70
	14	0.113	2.59	3.19
	SEM	± 0.037	± 0.763	± 0.619
Toluene	DAY 0	0.116	4.34	4.86
	7	0.103	1.43	2.96
	14	0.114	2.55	3.19
	SEM	± 0.009	± 0.880	± 0.388
Hexane	DAY 0	0.116	4.34	4.86
	7	0.120	1.86	2.28
	14	0.120	2.93	2.79
	SEM	± 0.012	± 0.712	± 0.564

The Chlorophyll a content of the microalgae (Chlorella vulgaris and Nitzschiapalea) showed persistent increase in chlorophyll a synthesis from day 7 to day 14 in the WSF of the various hydrocarbons. In (Anabaena flosaquae) apart from WSF of Xylene where there was an increase in Chlorophyll a synthesis in treatments cultures other WSFs resulted in decreased chlorophyll a from day 7 to day 14 of the experiment.

DISCUSSION

A wide range of hydrocarbons that contaminate the environment has been shown to be biodegraded in various extreme environments characterized by low or elevated factors such as temperatures, pH and salinity e.t.c. Most investigations on phytotoxicity and biodegradation of hydrocarbons have concentrated on the use of biodegrading microorganisms. Comparatively, what is known about the responses of microalgae to metabolism of hydrocarbons were data in the 70s and late 90s [19 and 20]. Scanty literatures exist on the subject in the 20s. This however is a recent addition to existing data. Hydrocarbon biodegradation can be significantly stimulated under favorable nutrient conditions. The increasing number of organism so far reported indicates that there is a growing interest in the commercial application of hydrocarbon degraders for biological, environmentally friendly treatment of polluted water. Microorganisms useful for bioremediation must survive and be active under in situ conditions. In this study: the following findings were obvious*C. vulgaris* showed growth stimulation at 10% - 50% concentration 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 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 in all concentrations of WSF of Benzene. *C. vulgaris* showed adverse effects on Photosynthetic pigment only at higher concentrations while *N. palea* shows no negative effects in Photosynthetic pigment in all concentrations (Table 4). The order of phytotoxicity of WSFs of hydrocarbons to the microalgae follows this pattern: Toluene > Xylene > Benzene > Hexane. Phytotoxicity was higher in WSF of aromatic hydrocarbons (Benzene, Xylene and Toluene) than the WSF of the aliphatic hydrocarbon (Hexane).The growth stimulating effect observed in the WSF of hexane could be due to high rate of evaporation of the toxic components of the WSF before the termination of the experiment. According to [28] oil extract stimulate algal growth and photosynthesis after the evaporation of toxic substances. Our results support these findings at low to mid concentration of WSF of Xylene (10% - 50%) for*C. vulgaris*and *A. flosaquae*,10% - 75% of Toluene and Hexane and 10% - 100% of Benzene. Our results support this finding in all concentrations (10% - 100%) for *N. palea*. The inhibition observed at 100% concentration of most WSFs by the various species could be due to the presence of high amount of inhibitory substances than other concentrations. This is in accordance with the work of [18]. In WSF of aromatic hydrocarbon Benzene, Toluene and Xylene), the principle effects observed include growth stimulation in low and mid concentrations of the various WSFs, higher inhibitory effect on growth rate, prolonged lag phase at the onset of the study and higher death rate of microalgae in higher concentration except *N.palea* were recorded. These responses were more pronounced in the aromatics than the aliphatic hydrocarbon (hexane). This indicates higher phytotoxicity properties in WSF of aromatics than those of aliphatic (hexane). Another possible reason may be due to increased productivity which induced a rise in the metabolite concentrations in the growth medium. This metabolites or secondary products are derived from the parent hydrocarbons when they are broken down via photo oxidation. In addition to being more toxic, aromatic compounds are generally more soluble than aliphatic compounds [18]. This probably accounted for the phytotoxicity trend on the test microalgae observed in this study being Toluene > Xylene > Benzene >Hexane which is their corresponding order of decreasing solubility. The inhibitory effects observed in this study at higher concentrations were more pronounced during the exponential growth phase. During this phase, the growth rate of treatment cultures was less than that of the control. However, at the end of the experiment, there was no significant difference ($p<0.005$) in the growth rate of microalgae in control and low concentration of treatment cultures. This could be attributed to nutrient deficiency in higher concentrations of treatment cultures. But, without detailed analyses of the nutrient content of the culture media at the end of the experiments, it is impossible to draw firm conclusions. Perhaps the most likely explanation may reside with the presence of more inhibitory substances associated with the various WSFs at higher concentrations. This finding is further supported by the work of [29] who noted that the water soluble fractions from four fuel oil showed considerably different inhibitory effect on growth of six microalgae namely (*Agmenellumquadruplicatum, Coccochloriselabens, Dunaliellatertiolecta, Chlorella autotrophica, Cylindrotheca*sp and *Amphora*sp). The inhibitory effect in various concentrations could also been occasioned by changes in growth medium due to photo oxidation and microalga photosynthetic activities. This view is corroborated by the findings of [27] who reported that hydrocarbons cause a disruption of the optimal physical state of the cytoplasmic membranes thus compromising the permeability of these membranes to hydrocarbons which in turn facilitate the entry and accumulation of hydrocarbons into the cells.

The decrease observed in photosynthetic pigment could be attributed to degeneration and break down of chlorophyll arising from bleaching effect of the various fractions at high concentrations. This finding is corroborated by [5 and 10] who reported a reduction in photosynthetic rate and pigment composition of microalgae on exposed to higher concentration of oil and its soluble fractions. Unavailability of nutrient

at higher concentration could be another reason for poor growth response and adverse effect on growth parameters observed in most microalgae at higher concentrations in this study. The inhibitory effect could also be due to disruption of cell metabolism such as (changes in photosynthesis, growth rate and pigment structure) which affects primary production. This observation is similar to the findings of [13] who reported significant decrease in chlorophyll *a* content as the concentration of fuel oil increased in their work titled fuel oil effect on the population growth, species diversity and chlorophyll *a* content of fresh water microalgae.

CONCLUSION

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 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).

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