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RESPONSE OF MICROALGAE FROM TIDAL AND NON-TIDAL CREEKS IN LAGOS TO CRUDE OIL CONTAMINATION.

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ABSTRACT

The importance of microalgae in oil polluted environments has received greater attention in recent times and this has led to several researches on potentials of algae. In the present study, samples of water were collected from Mangrove (tidal) and Ogbe creek (non-tidal) in University of Lagos. Different concentrations of crude oil were added to appropriate volume of water samples such that the mixture of the two was 100ml. The optical density of the samples were measured every other day for 14 days using a spectrophotometer at 680nm. Samples were analysed before and after experiment. Light microscopy identification revealed *Chroococcus*, *Amphipleura*, *Pinnularia*, *Navicula* and *Oscillatoria* as surviving species. Although there were fluctuations in the growth rate of both samples, the mangrove sample showed greater and faster utilization than Ogbe creek. This study revealed that degradation rate is subject to environmental conditions, amount of oil tolerant phototropic micro-organisms and the availability of nutrients.

Keywords: Biodegradation, Creeks, Crude oil, Environment, Microalgae,

INTRODUCTION

Petroleum is perhaps the most important substance consumed in modern society. It provides not only raw materials for the ubiquitous plastics and other products, but also fuel for the energy, industry, heating and transportation. Crude oil, a mixture of complex organic and inorganic compounds, whose composition can vary from one field to the next, within the same field and

even at different times and depth within the same drill hole. Industrialization has long been accepted as a hallmark of civilization. However, the fact remains that industrial emanations have been adversely affecting the environment (Rajasulochana *et al*., 2009). The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the ecosystem (Gutnick and Rosenberg, 1977). Addition of oil to the soil as a deliberate policy of waste disposal also leads to contamination (Flowers *et al.*, 1984). Nigeria has coastline of approximately 853km facing the Atlantic Ocean, this coastline lies between latitude 4^o 10' to 6^o 20' N and longitude 2^o 45' to 8^o 35' E. In 1956, Royal Dutch Shell discovered crude oil at Oloibiri, a village in the Niger Delta, and commercial production began in 1958. Since the discovery of oil in Nigeria in 1956, the country has been suffering the negative environmental consequences of oil exploration and exploitation. According to the Department of Petroleum Resources (DPR), between 1976 and 1996 a total of 4647 incidents resulted in the spill of approximately 2,369,470 barrels of oil into the environment. In addition, between 1997 and 2001, Nigeria also recorded a total number of 2,097 oil spill incidents (Nwilo and Badejo, 2005). In 1998, 40,000 barrels of oil from Mobil platform off the Akwa Ibom coast were spilt into the environment causing severe damage to the coastal environment (Egberongbe *et al.*, 2006). Sabotage is another major cause of oil spillage in the country.

Bioremediation is the utilization of microorganisms to remove pollutants from the environment; it is an acceleration of the natural fate of biodegradable pollutants and hence can be regarded as a green solution to oil pollution (Abhijit and Kakoli, 2004). Bioremediation has a great potential for destroying environmental pollutants (Song *et.al.,* 1990). The use of inexpensive equipment, environment friendly nature and simplicity of the process are some of its advantages over other remedial alternatives such as physical and chemical treatments. Organic pollutants in the aquatic environment are subject to biodegradation by a range of naturally occurring microorganisms, but studies have concentrated, almost exclusively, on the role of bacteria (Dagley, 1978) and fungi (Middlehoven, 1993) in the degradative processes. Some species of algae are capable of heterotrophic growth on organic carbon sources (Neilson and Lewin, 1974). One way of investigating the biodegradation of organic pollutants by algae is to encourage the cells to grow in the presence of the pollutant. It is believed that some groups of algae can at most initiate the biodegradation of hydrocarbons by oxidizing them to components of lower molecular weight or by the transformation of petroleum hydrocarbons to more polar compounds of a carbon equal to the parent compound (Al-Hassan *et al.,* 1994).

The aim of this study is to investigate the microalgae that will survive in the oil polluted samples for their possible use in biodegradation of crude oil contaminated waters.

MATERIALS AND METHODS

Description of Study sites

The Mangrove (Site A): This site has coordinates of 06^o 30' 53.6'' N; 003^o 24' 16.8''E. It is directly opposite the Lagos Lagoon and steadily inundated by water from the

Lagoon. The study site is covered by *Nymphea lotus* and *Eichhornia crassipes* Mart. Solm. The water appeared cloudy giving off characteristic odour. The surrounding vegetation includes the *Rhizophora* sp., *Avicennia germinans*, *Paspalum vaginatum*, *Drepanocarpus lunatus*, and *Acrosticum aureum.* The soil is waterlogged and soft with several pneumatophores growing through the water, while the prop roots of the *Avicennia* sp. are well represented. The habitat supports aquatic organisms like fish and crabs as burrowing holes are abundant on fairly-dried part of the soil.

Ogbe Creek *(*Site B): with coordinates of 06^{o} 30' 48.4'' N; 003^{o} 23' 37.4'' E, found along under bridge that connects the University of Lagos, Akoka second gate axis and the Medical Centre/Residential Areas. This creek has a small pedestrian bridge just above it which enables people to observe the habitat more closely. The water surface is largely covered by *Acrosticum aureum* and has abundant fringing forest around it with such species as *Alchonea cordifolia*, *Ficus sur*, *Ipomoea asarifolia*, *Panicum maximum*, *Ludwigia erecta*, *Cyathea dnegeii* and *Ipomoea spp*. People have been seen to fish in this creek and at other times, crabs were sought after in the surrounding creeks.

Collection of samples

Water samples were collected on 15th of March 2011 at the two sites representing the tidal (Mangrove) and non-tidal (Ogbe creek) creeks. Two replicates of sample were collected and taken to the laboratory for physico-chemical and biological analysis. For biological samples, each of the water samples from the study sites were divided into two separate containers. The first container was allowed to settle down for 24 hours and decanted to get about 400ml of each sample while 100ml of each water sample was measured from the second container for the purpose of microscopic examination. This was necessary to observe the algal population in the samples before the experiment. Microalgae observed were appropriately identified and documented using manuals and monographs. In the laboratory, sixteen (16) 250ml properly labelled conical flasks were arranged on the bench. The crude oil sample used for the experiment is of Forcados type. Water sample was gently shaken and measured into the previously labelled conical flasks. The measurement was done in a way that both the water sample and the crude oil make 100ml for each conical flask (Table 1).

Table 1. Water samples and crude oil combination ratio.

Water Samples Concentrations	Crude Oil Concentrations
99.0ml	1.0ml
97.5ml	2.5ml
95.0ml	5.0ml
92.5ml	7.5ml
90.0ml	10.0ml
87.5ml	12.5ml
85.0ml	5.0ml
Control (100ml)	Nil

Before the addition of the pollutant, the optical densities of the samples were measured using Cecil 2041 photospectrometer at 680nm. After the addition of the crude oil into the conical flasks, the flasks were corked with cotton wool to prevent contamination by foreign organisms. and then arranged

close to the windowpane for maximum exposure to sunlight. It was exposed to 16hrs/8hrs of light and darkness at room temperature of $\pm$ 26^{o}C. Samples were gently shaken daily to dislodge any attached organisms. Optical density was measured using Cecil 2041 Photospectrometer. After the 14 days, crude oil was carefully removed using micropipette and each sample was observed using Light microscope. This was done to observe surviving algae species at end of the bioassay.

Physico-chemical analysis

The Total Dissolve Solids and Conductivity were determined using an Adwa AD31 & AD32 Instrument, a professional IP67 waterproof meter. Salinity was measured using a handheld refractometer while air temperature was measured with a mercury-in-glass thermometer. The readings were recorded in degree

Celsius (^{0}C). Hydrogen ion concentration was determined using Philip pH meter while Dissolved Oxygen was estimated using the titrimetric (Iodometric) method and Nitrate-nitrogen was determined using Colorimetric method (APHA/HACH DR 2010) Colorimeter with internal standard. Phosphate-Phosphorus was estimated using Stannous chloride method while Sulphate was determined using the turbidimetric method (APHA, 1998).

RESULTS

Total dissolved solid for the Mangrove and Ogbe creek samples were 8.57mg/L and 0.10mg/L while conductivity values were 16.48mScm^{-1} and 0.21mScm^{-1} and the salinity values were 22‰ and 2‰ respectively. The air temperature and pH of the Mangrove site were 29.0^{o}C and 7.6 while Ogbe creek recorded 28.0^{o}C and 7.2 while the Dissolved oxygen values were 4.7mg/L and 7.1mg/L for Mangrove and Ogbe creek. Nitrate-nitrogen and phosphate-phosphorus values of 96.0mg/L and 6.40mg/L were recorded for Mangrove sample while Ogbe creek recorded 0.17mg/L and 0.01mg/L respectively. The Sulphate concentration of the Mangrove sample was 340.0mg/L and Ogbe creek had 2.30mg/L (Table 2).

Table 2. Physico-chemical analysis of Mangrove and Ogbe creek samples.

Parameter	Mangrove Sample	Ogbe Creek Sample

Temperature (^oC)	29.00	28.00
Conductivity ($mScm^{-1}$)	16.48	0.21
Salinity(%)	22.00	2.00
pH	7.60	7.20
Dissolved Oxygen (mg/L)	4.70	7.10
Total Dissolved Solid (mg/L)	8.57	0.10
Nitrate-Nitrogen (mg/L)	96.00	0.17
Phosphate-Phosphorus (mg/L)	6.40	0.01
Sulphate (mg/L)	340.00	2.30

Biological samples

The growth rates measurement (at 680nm) of the samples fluctuated in both the Mangrove and Ogbe creek samples (Table 3). The optical densities of the Mangrove and Ogbe Creek samples before the experiment were 0.864C and 0.030C respectively at 680nm. The 0.1ml sample for the Mangrove had its lowest growth rate as 0.031C (day 4) and the highest rate at 0.387 (day 6) while the 0.1ml for Ogbe creek had its lowest 0.021C and highest 0.227C growth rates at days 2 and 14 respectively. The 2.5ml sample for the Mangrove recorded its lowest and highest growth rates as 0.076C (day 4) and 0.835C (day 12) respectively with optimum growth around days 10 and 12 (Fig.1). Ogbe creek sample had the lowest value (0.010C) and highest 0.281C growth rates on days 2 and 12 respectively. The 5.0ml Mangrove and Ogbe samples followed same pattern as the lowest and highest values were recorded in same day (Fig.2) also Ogbe 5.0ml followed same pattern as the 2.5ml concentration (Fig.3). For 7.5ml samples, days 8 and 6 recorded 0.035C and 0.748C (Mangrove) while 0.019C and 0.428C were recorded for days 2 and 14 (Ogbe creek) as lowest and highest growth rates. Mangrove sample recorded same days for the 7.5ml and 10ml but with different values for the 10.0ml sample, 0.031C (day 8) and 0.545C (day 6) were whereas Ogbe Creek had, for the same concentration, 0.044C (day10) and 0.290C (day 12) as its values growth rates.(Fig.4) The lowest and optimum growth rates values for the 12.5ml sample of Mangrove was 0.034C (day 10) and 0.444C (day 2) while the values for Ogbe creek were 0.035C (day 10) and 0.536C on day 8 (Fig.5). The control sample as compared to the growth rate of polluted samples is represented in Figures 7 and 8. Phytoplankton observed in the water samples before and after the experiment were presented in Tables 4 and 5.

Table 3: Growth rates comparison of both Mangrove and Ogbe creek samples for the period of bioassay (Mangrove = MGV)

	0.1ml	2.5ml	5.0ml	7.5ml	10.0ml	12.5ml	15.0ml	Control

Days	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe	MGV	Ogbe
17/03/11	0.14C	0.21C	0.28C	0.01C	0.19C	0.03C	0.26C	0.02C	0.12C	0.06C	0.06C	0.10C	0.20C	0.05C	0.04C	0.08C
19/03/11	0.03C	0.05C	0.08C	0.04C	0.16C	0.27C	0.13C	0.06C	0.43C	0.29C	0.44C	0.15C	0.21C	0.04C	0.11C	0.08C
21/03/11	0.39C	0.15C	0.13C	0.24C	0.39C	0.43C	0.75C	0.09C	0.55C	0.22C	0.07C	0.26C	0.11C	0.27C	0.04C	0.06C
23/03/11	0.21C	0.03C	0.43C	0.03C	0.04C	0.05C	0.04C	0.09C	0.03C	0.07C	0.26C	0.54C	0.05C	0.06C	0.10C	0.06C
25/03/11	0.13C	0.14C	0.79C	0.05C	0.06C	0.07C	0.04C	0.04C	0.04C	0.04C	0.03C	0.04C	0.70C	0.06C	0.12C	0.04C
27/03/11	0.27C	0.10C	0.84C	0.28C	0.61C	0.69C	0.09C	0.07C	0.30C	0.29C	0.11C	0.14C	0.21C	0.34C	0.18C	0.06C
29/03/11	0.05C	0.23C	0.11C	0.19C	0.30C	0.56C	0.72C	0.43C	0.06C	0.24C	0.43C	0.32C	0.09C	0.18C	0.03C	0.08C

Table 4. Phytoplankton composition of the sample before the addition of the pollutant

Division	**Class**	**Order**	**Species**
Bacillariophyta	Bacillariophyceae	Naviculales	*Navicula placenta* Ehr. *Navicula crucicula* (W.Sm) Donkin *Amphipleura pellucida* Kutz *Pinnularia braunii* (Grun.) Cleve *Pinnularia subcapitata* (Grun.) Cleve *Cymbella tumidula* Grunow
		Thalassiophysales	*Amphora lineolata* Ehrenberg
		Eunotiales	*Actinella punctata* Lewis
		Fraginariales	*Ulnaria ulna* (Nitzsch) Ehrenberg
Chlorophyta	Chlorophyceae	Desmidiales	*Closterium gracile*`Breb
Cyanophyta	Cyanophyceae	Chroococcales	*Chroococcus disperses*
		Oscillatoriales	*Oscillatoria lacustris* Geitler *Planktothrix planctonica* Elenkin *Schizothrix arenaria* (Berk.) Gom

Table 5. The surviving microalgae in the crude oil polluted samples after the period of 14 days.

Concentration	Mangrove Samples	Ogbe Creek Samples

0.1ml	*Chroococcus dispersus, Navicula crucicula, Navicula placenta, Pinnularia subcapitata, Pinnularia braunii, Amphipleura pellucida*	*Chroococcus dispersus , Amphipleura pellucida*
2.5ml	*Chroococcus dispersus , Amphipleura pellucida, Navicula placenta, Navicula crucicula ,Pinnularia subcapitata, Oscillatoria lacustris*	*Chroococcus dispersus*
5.0ml	*Chroococcus dispersus, Navicula placenta, Navicula crucicula, Oscillatoria lacustris.*	*Chroococcus dispersus, Navicula placenta*
7.5ml	*Chroococcus dispersus, Navicula placenta, Pinnularia braunii.*	*Chroococcus dispersus, Pinnularia braunii*
10.0ml	*Chroococcus dispersus, Navicula placenta*	*Chroococcus dispersus,*
12.5ml	*Chroococcus dispersus , Navicula placenta, Pinnularia braunii*	*Chroococcus dispersus*
15.0ml	*Chroococcus dispersus, Navicula placenta, Pinnularia braunii, Amphipleura pellucida*	*Chroococcus dispersus*

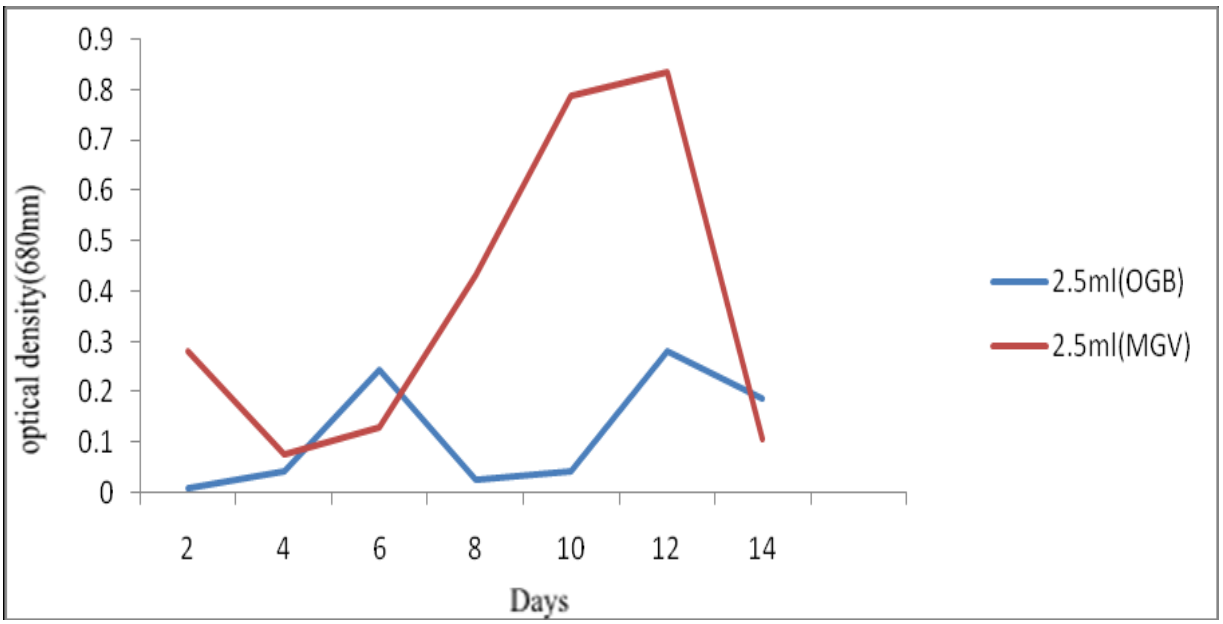

Figure 1. Comparison of growth rates of microalgae in 2.5ml concentration of crude oil in Mangrove and Ogbe creek samples.

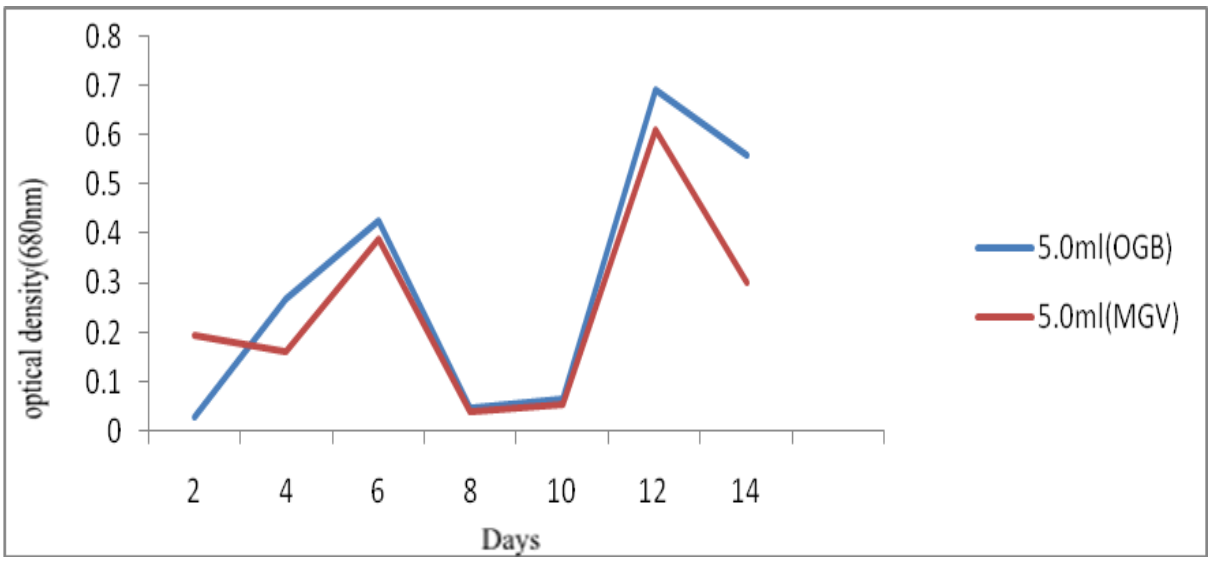

Figure 2. Comparison of growth rates of microalgae in 5.0ml concentrations of crude oil in Mangrove (MGV) and Ogbe creek (OGB) samples.

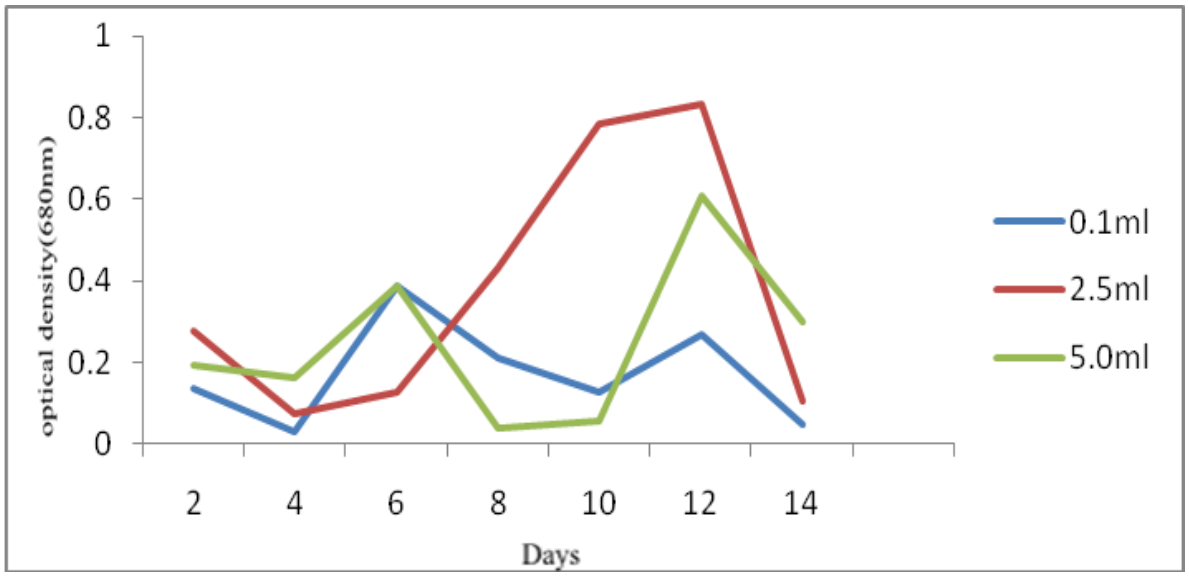

Figure 3. Comparison of growth rates of microalgae in 0.1ml, 2.5ml and 5.0ml concentrations of crude oil in Mangrove samples.

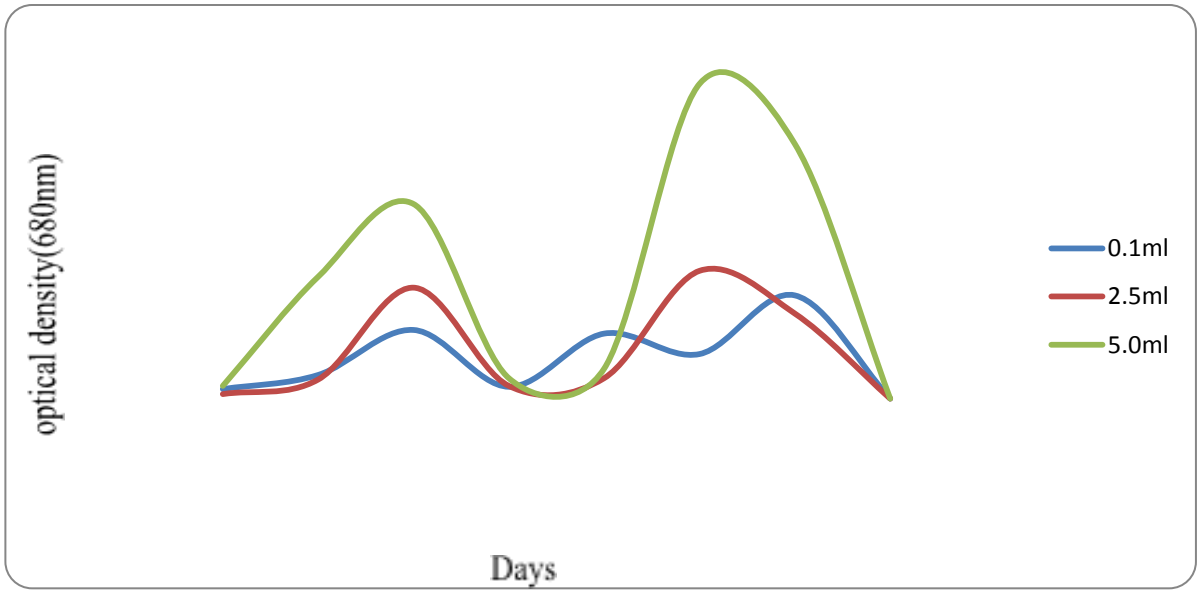

Figure 4. Comparison of growth rates of microalgae in 0.1ml, 2.5ml and 5.0ml concentrations of crude oil in Ogbe creek samples.

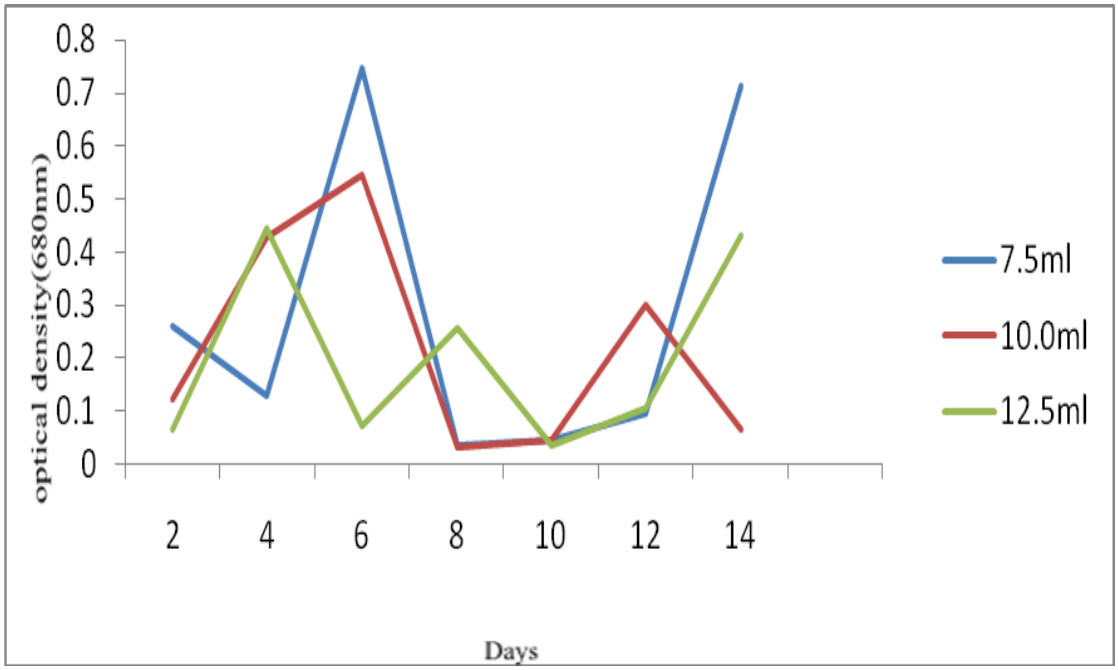

Figure 5. Comparison of growth rates of microalgae in 7.5ml, 10.0ml and 12.5ml concentrations of crude oil in Mangrove samples.

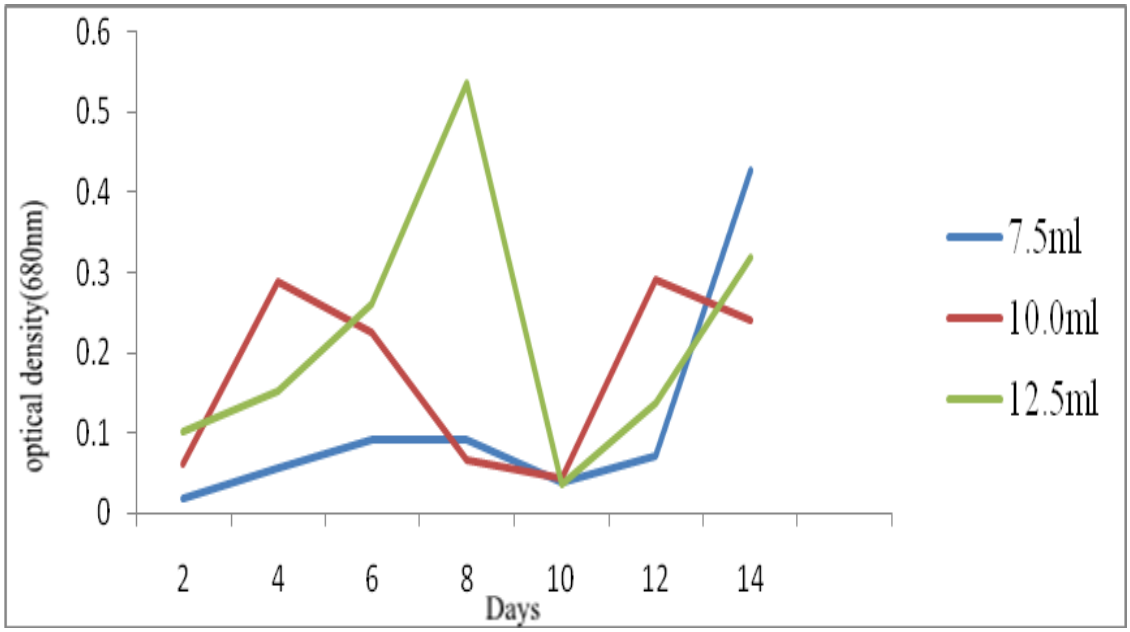

Figure 6. Comparison of growth rates of microalgae in 7.5ml, 10.0ml and 12.5ml concentrations of crude oil in Creek samples.

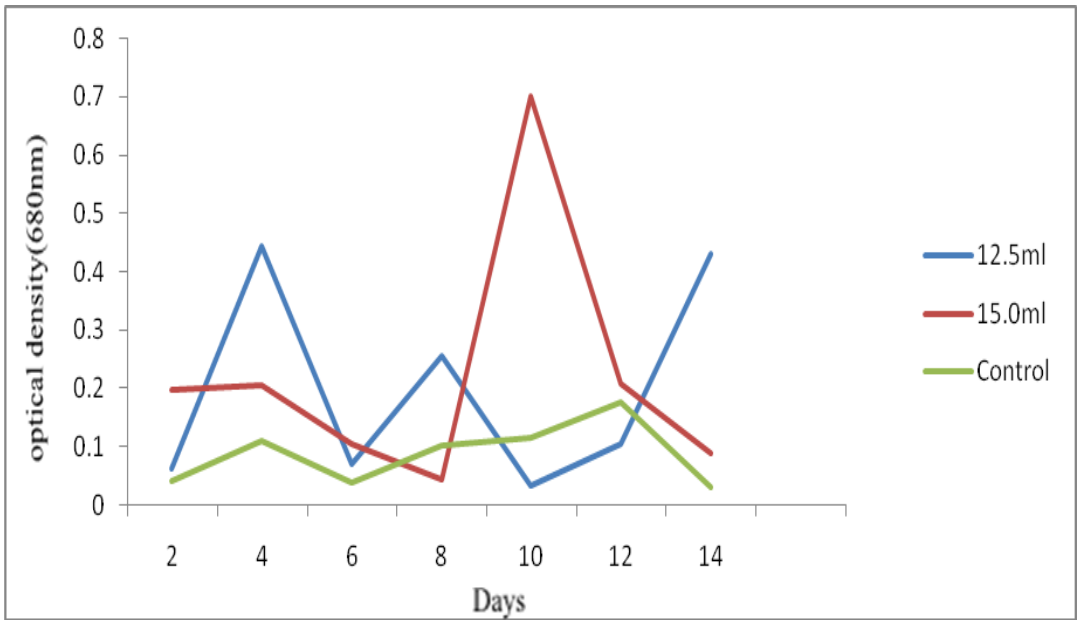

Figure 7. A comparison of growth rates of microalgae in Control sample, 12.5 ml and 5.0ml crude oil concentrations in Mangrove samples.

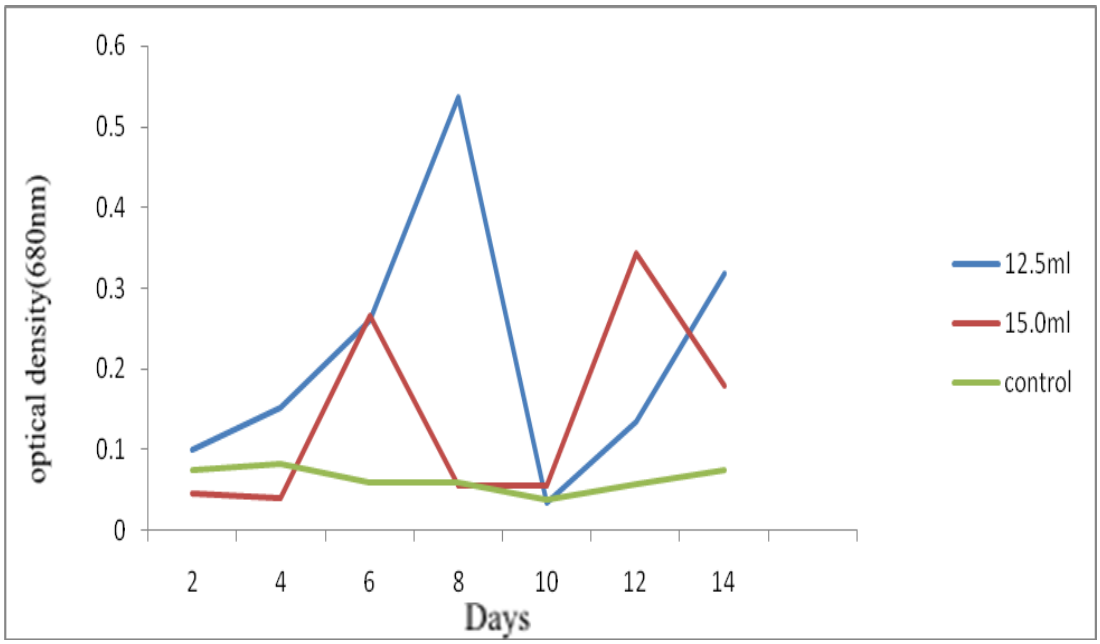

Figure 8. A comparison of growth rates of microalgae in control sample, 12.5ml and 15.0ml crude oil concentrations in Ogbe creek samples.

DISCUSSION

The result of this study shows that indigenous microalgae from the tidal and non-tidal creeks are

capable of utilizing crude oil for their growth. The crude oil provides them with rich carbon source, which was utilized for the proliferation of the microalgae population and further degradation of the oil. However, the rate of degradation is subject to the prevailing environmental conditions, amount of oil-tolerant phototropic microorganisms (comprising green algae and cyanobacteria) and the amount of nutrients available for utilization. In this present study, the optimum growth values of the Mangrove sample for all concentrations (except 5.0ml and 12.5ml) are greater when compared to those of corresponding Ogbe Creek samples. Sorkhoh et al. (1992) reported that oil polluted sites covered by cyanobacterial mats showed more recovery signs than those uncovered, indicating their capacity to degrade oil and Al-Thukair et al. (2007) found that cyanobacteria play a major role in oil biodegradation in the coastal intertidal zone. A reason for the abundance of *Chroococcus dispersus* is given by (Belma and Sahlan, 2009) as the ability of cyanobacteria to inhabit various aquatic habitats, especially polluted and heavily polluted ones where they have a wide distribution and may be dominating microflora in the systems. According to Dibble and Bartha, 1979, using an indigenous microorganism ensure that the organisms have a higher tolerance to the toxicity of hydrocarbons and are resistant to variations in the environment. The 7.5ml and 12.5ml concentrations for both Mangrove and Ogbe Creek samples appear to be the most suitable samples as their optical densities show sign of rising on day 14 of the experiment. Importantly, it is extremely rare to find a single microorganism that is capable of completely degrading a pollutant or a mixture of xenobiotics under environmental conditions (Alexander, 1994).Since biodegradation activities observed during the experiment was by different algae species, culturing techniques can be used to isolate individual alga species so as to evaluate the biodegradation potentials of each species when a homogenous culture is used.. Further studies can be carried out on factors that may be responsible for transient increase in toxicity of cultures and how it can be controlled. The importance of using algae as biodegradation tool is that algal technology is very economical and safe for the environment (Kamaleswari *et al*., 2007). The use of algal technology probably will help in solving part of oil spill problems in Nigeria.

REFERENCES

Abhijit, M. and Kakoli, B. (2004). Marine Microbiology. Narendra Publishing House, Delhi, India.159pp.

Alexander, M. (1994). Biodegradation and bioremediation. Academic Press, NewYork. 692pp.

Al-Hasan, R., Sorkhoh, N., Al-Bader, D. and Radwan, S. (1994). Utilization of hydrocarbons by cyanobacteria from microbial mats on oily coasts of the Gulf. Applied Microbiology Biotechnology 41: 615-619.

Al-Thukair, A., Abed, R. and Mohamed, C. (2007). Microbial Community of Cyanobacterial mats in the intertidal zone of oil-polluted coast of Saudi Arabia. Marine Pollution bulletin 54:173-179.

American Public Health Association (1998). Standard methods for the examination of water and water, 20th ed. American Public Health Association Incorporated, New York. 1270pp.

Belma, A. and Sahlan, O. (2009). Toxicity of

herbicides to Cyanobacteria Isolates. Journal of Environmental Biology 30: 381-384.

Dagley, S. (1978). Microbial catabolism, the carbon cycle and environmental pollution. Naturwissenschaften 65:85-95.

Dibble, J. and Bartha, R. (1979). The effect of environmental parameters on the biodegradation of oily sludge.Applied Environmental Microbiology 37:729-739.

Egberongbe, F.O., Nwilo, P.C. and Badejo, O.T. (2006). Oil spill Disaster Monitoring Along NigerianCoastline. 5th FIG Regional Conference, Accra, Ghana.

Flowers, T.H., Pulford, I.D. and Duncan H.J. (1984). Studies on the breakdown of oil in soil. Environmental Pollution (Series B.) 8: 71-82.

Gutnick, R. (1977). Oil tankers and pollution: A microbial approach. Annual Review of Microbiology 31: 379-396.

Kamaleswari, J., Murugesan, S. and Sivasubramanian, V. (2007). Screening of freshwater algae for phycoremediation potentialities of industrial effluents and wastewater. Ecology Environment and Conservation. 13: 697-701.

Middlehoven, W.J. (1993). Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeast-like fungi. Antonie van Leeuwenhoek, 63: 125-144.

Neilson, A. and Lewin, R. (1974). Uptake and utilization of organic carbon by algae. Phycology 13: 229-257.

Nwilo, P.C. and Badejo, O.T. (2005): Oil Spill Problems and Management in the Niger Delta. International Oil Spill Conference, Miami, Florida, USA.

Rajasulochana, R., Dhamotharan, R., Murugesan, S. and Ramachandra M. (2009). Bioremediation of Oil Refinery Effluent by Using *Scenedesmus obliquus*. Journal of American Science 5:17-22.

Song, H., Wang, G. and Bartha, R. (1990). Bioremediation potential of terrestrial fuel spills. Applied Environmental Microbiology 56: 641-65.

Sorkhoh, N., Al-Hasan, R. and Radwan S. (1992). Self-cleaning of the Gulf. Nature 3: 59: 109.