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Remediation Of A Crude Oil Polluted River In B- Dere In Ogoniland, Rivers State Using *Chaetomorpha* And *Nostoc* Species

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Abstract
Aim : *To Investigate The Ability of Chaetomorpha and Nostoc Species in Remediating Crude Oil Polluted Rivers in Ogoniland.*
Study Design*: This Study Employs Experimental Design and Statistical Analysis of Data And Interpretation.*
Place and Duration of Studies*: crude oil polluted water samples were collected from B-dere in Gokana local government area in rivers state, where crude oil has polluted their environment. Using sterile bottles water samples were collected, preserved in an iced pack and transported to the microbiology laboratory of the rivers state university within 6 hours of collection and analysis was carried out immediately.*
Methodology*: Standard microbiological techniques were used : single cell technique as adopted by Williams and Youngtor (2017) was used in the isolation of Nostoc and Chaetomorpha species from unpolluted water samples using BG11 Agar and Nutrient Agar containing Nystatine and Chloramphenicol. Bioremediation set up was done by further polluting water sample with various concentrations (%) of crude oil: 4, 40 and 80, respectively in 300ml sterilized water sample in conical flasks, then augmented with 5ml of the organisms and a control experimental setup without organisms for duration of 14days.*
Results*: The result indicate that there was a progressive increase in the total Chaetomorpha and Nostoc count from all the setup from day 0 to day 14, the consortium setup had the highest count of the organisms, followed by the Chaetomorpha set up ,the the Nostoc set up and then control had the lowest count. The optical density monitored in this study showed that there was steady increase in the optical densities of the crude oil polluted water samples. The mixed culture had the highest absorbent rate due to the presence of chlorophyll, nitrogen, phosphorus and other physicochemical parameters. Percentage of remediation ranged from 4%; control 16.56<Chaetomorpha 23.86, Nostoc 25.24 < and Nostoc+ Chaetomorpha 34.35. 40%; control 14.57<Chaetomorpha 25.70Nostoc 27.13 < and Nostoc+ Chaetomorpha 32.60, 80% control 16.14<Chaetomorpha 25.37, Nostoc 26.16 < and Nostoc+ Chaetomorpha 32.32 which shows that set-up with combined cyanobacteria species had the highest remediation percentage. This may be due to synergetic effect of organisms. The control set-ups in all the crude oil concentrations had the lowest remediation percentage which revealed that all the isolates have the capacity to degrade crude oil either as individual or in consortium.*
Conclusion: *The results from this study revealed that Cyanobacteria species (Chaetomorpha and Nostoc species) are useful bioremediation tools in crude oil polluted sites. Employment of this method in large scale bioremediation of crude oil pollution is essential. Since there is significant difference among the samples of the Cyanobacteria in their bioremediation percentage, it means that their effectiveness and remediation potential varied in remediating the environment. This work is also coherent with previous studies that showed abattoir effluent and aquaculture water as sources of essential nutrients and media for the cultivation of Cyanobacteria.*
Keywords*: Bioremediation, Cyanobacteria Chaetomorpa,Nostoc , Crude Oil Pollution.*

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I. Introduction

Recurrent oil spill incidence has become a trouble to ecological protection efforts. The associated effects of this pollution on the physical, chemical and biological constituents of the environment are in diverse forms (Nrior *et al*., 2017). Oil spillage generally contaminates the life support system which includes soil, air and water; affect their productivity and other biological processes such as biogeochemical cycle in the ecosystems (Pearl and Pall, 2012). Water which harbor microorganisms serve as one of the dynamic sites of biological interactions in nature. Many of the biochemical reactions that enhance mineralization of organic matter and nutrition in aquatic ecosystems that are mediated by organisms are affected by the degradation of the environment. In this respect, microbial activities are adversely affected and so, aquatic plant growth and mineralization of pollutants are also impaired (Williams and Obemeile, 2014). The damage to the ecosystems has negative affect on the socio-economy and livelihood of the people. The use of conventional synthetic

remediation formulations in the remediation of oil crude polluted environments are costly and could pose adverse effect on the normal flora and fauna of the environment.

Bioremediation is the use of natural processes to remove toxic chemicals and pollutants in the environment. Use of Microalgae/Cyanobacteria as a biological agent in bioremediation is a unique method because it is a self-sustaining cycle. To oxidize contaminants into less harmful metabolites, algae extract and utilize oxygen from its surrounding environment; these metabolites include CO_2 and H_2O. For growth, algae use photosynthesis, which requires carbon dioxide (CO_2) and water (H_2O). Photosynthesis, in turn, releases oxygen that algae can employ for further contaminant oxidation, thus repeating the cycle (Williams and Agunkwo, 2018).

Bioremediation of crude oil polluted aquatic ecosystems is a method that employs biological agents to depollute crude oil contaminated water bodies. This method (Bioremediation) is considered as an efficient and environmentally safe method because it poses no adverse effect on the environment and is also an inexpensive system for decontamination of polluted ecosystems (Yoo *et al.,* 2001). Petroleum hydrocarbons are foremost pollutants of the aquatic environments (Williams and Obomeile, 2014). All the exploration, production, transportation and refining of oil, handling of refined product and management of oily waste activities in the petroleum industries poseserious threats to human (Kannan, 2006). The possibility of preventing oil spills and complete remediation of contaminated systems is a major environmental concern.

The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 200,000-800,000 species in many different genera exist of which about 50,000 species are described by (Vidali, 2001). Over 15,000 novel compounds originating from algal biomass have been chemically determined. Most of these microalgae species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols (Pearl and Paul, 2012).

Bioremediation is an option that offers the possibilities to destroy or renders harmless, various contaminants using natural biological activities (Vidali, 2001). Bioremediation involves three principal approaches namely, natural attenuation (reliance on natural biodegradation activities and rates), which is sometimes called intrinsic bioremediation; bio stimulation (stimulation of natural activities by environmental modifications such as fertilizer addition to increase rates of biodegradation); and bioaugumentation (addition of exogenous microorganisms to the hydrocarbon-impacted ecosystem to supplement the existing microbial population). These three principles for in-situ biodegradation have been applied several times at pilot and field scale levels with varying degree of success (Chikere *et al.,* 2009, Eziuzor and Okpokwasili, 2009 Gertle *et al*., 2009, Kaplan and Kitts, 2004).

The control of bioremediation processes is a complex system of many factors. These factors include the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population, the environmental factors (type of soil, temperature, pH, the presence of oxygen and nutrients) (Ijah and Antai, 2003).

II. Materials And Methods

Sample Collection

Crude oil Polluted water sample was collected from B-dere in Gokana L.G.A and unpolluted water sample was collected from Luubaara in Khana L.G.A, both in Ogoni, Rivers State. The sampling points are about 25 kilometers away from each other. The water samples were taken to the Microbiology laboratory, Rivers State University, Port Harcourt for analysis in an ice pack within 6 hours of collection.

Microbiological Analysis

Isolation and Identification of Microalgae

Single cell technique as adopted by Williams and Youngtor (2017);Williams and Chibuike (2017);Williams and Agunkwo(2018)was used for the isolation of the Microalgae in the sample. Using one milliliter (1ml) sterile Pasteur pipette, an aliquot of the sample was placed on a clean glass slide, inserted on the light microscope, covered with cover slip and examined under x10 magnification of the light microscope and refocused at X40 magnification until the microalgae species were properly identified by their morphological characteristics. The two water samples were examined, but there were no visible microalgae in the polluted water due to the pollution. The water samples were also plated directly on BG11 agar and nutrient agar containing Nystatin and Chloramphenicol and incubated for 7 days (1 week) at 30°C (Williams and Agunkwo, 2018). After incubation, only the unpolluted water yielded growth of various microalgae.

The microalgae that grew on the respective media were identified as *Nostoc* and *Chaetomorpha* species. The two identified species of microalgae were aseptically inoculated into BG11 broth and kept where they could receive sun light for 18 hours per day for one week to enable growth and multiplication.

Media Used,

BG11 is the medium used for cultivation of microalgae. It consists of two solutions: A and B.

Solution Aconsists of the following:
$NaHCO_3$ (11.61g), $NaCo_3$ (3.53g), K_2So_4 (0.5g) Distilled water (500ml)
Solution B consists of the following: $NaNo_3$ (5g),K_2So_4 (1g),$NaCl_2$ (1g), $MgSo_4$ + H_2O(0.2g) $CaCl_2$(0.04g) EDTA (0.5M)1ml,Distilled water500ml.
Solution A and B were mixed after autoclaving at 121◦C at 15 psi for 15 minutes.

Nutrient Agar

This was prepared according to the Manufacturer's instruction of dispensing 28g of nutrient agar into 1000ml of distilled water. Mass/Volume relationship was used to compute actual required measurements. The mixture was mixed vigorously and then sterilized by autoclaving at121 ◦C at 15psi for 15minutes. Antibiotics (Chloramphenicol) and Nystatin (an anti-fungal agent) were mixed in the media and allowed to cool for about 10seconds before pouring into the petri dishes. Chloramphenicol was added to inhibit bacterial growth while Nystatin was added to inhibit fungal growth. The media was allowed to cool and solidify and dried in a hot air oven before inoculation with the river water (aqua culture), (Williams and Agunkwo, 2018).

Inoculation

One millimeter (1ml) of the sample was dispensed into 9ml of normal saline. An aliquot of the sample was transferred using a sterile pipette into the agar plate which was inoculated for one week. A pure culture of the organism was obtained by repeated sub-culturing on nutrient agar by using the streak plate method and incubated for 1 week until heavy growth was obtained. For each species, as well as the mixed culture, nine flasks (100 ml of sterilized modified Cyanobacteria medium in 125ml, 250-ml and 500-ml conical flasks) were prepared and sterilized. Each flask was inoculated with 5 ml of the 1-week dense individual Cyanobacteria suspensions, or with multiple species in the case of the mixed culture, incubated under the previously mentioned conditions and left to reach mid-late log phase of growth (E10 days), (Williams and Agunkwo, 2018,;Williams and Chibuike, 2017; Williams and Youngtor, 2017).

Bioremediation set-up

Bioremediation set-ups were made with the polluted water samples. The water was further polluted with various concentrations (%) of crude oil: 4, 40, and 80 in 300ml of sterilized water sample in a conical flask then augmented with 5ml of microalgae in the BG11 broth except for the control which was not augmented with any organism. The abattoir effluent (which serves as a growth nutrient) (4,8 and 12%) was transferred into twelve (12) conical flasks containing 300ml of water polluted with crude oil in a ratio of 4:40:80 and was coded as O-C for *Chaetomorpha*, and N-S for *Nostoc*, OC1 – ON3 for mixed culture. Appropriate controls were also set up, containing only 4% crude oil polluted river (Control Oa), 40% crude oil polluted river (Control Ob) and 80% crude oil polluted river (Control Oc). The set-ups were kept close to the window for natural source of light (sunlight) to penetrate. The flasks were intermittently shaken three times daily to enhance growth by preventing sedimentation of the microalgae and avoiding thermal stratification which involves gas exchange between culture medium and air to ensure that cells of the population were equally exposed to light and nutrients.

Monitoring of the Bioremediation set-up
Percentage (%) biodegradation evaluation
The percentage (%) biodegradation rate was calculated using the formula adopted by Nrior *et al.,* (2017) as follows:
Step 1:
Amount of hydrocarbon remediated equals Initial concentration of total hydrocarbon content (Day 0) minus final concentration of total hydrocarbon content at the end of experiment (last day).
Step 2:
Percentage (%) bioremediation equals amount of total hydrocarbon remediated divided by initial concentration of total hydrocarbon content (Day 0 or 1) multiplied by 100.
Thus; BC =IC – FC
Bx = IC – IO
Where, BC=Amount of total hydrocarbon degraded
IC=Initial concentration of total hydrocarbon content (Day 0)
FC= Final concentration of total hydrocarbon content at the end of experiment (Last day)
IO= Initial concentration value of Control on day 0
Bx= Actual amount of heavy metal in test medium

$$\% \text{ Bioremediation} = \frac{BC}{Bx} \times 100$$

Total Cyanobacteria Counts

An aliquot from 10^{-2} dilution of each sample, after serial dilution of all samples was inoculated in duplicate on Nutrient and BG11 agar, plated using spread plate technique as described by (AOAC, 2000) and incubated at room temperature (28 – 30°C) with a light source for 5 to 7 day. After incubation, colonies that grew on the plates were counted and average taken then expressed as colony forming unit per milliliter using the formula as adopted by (Nrior *et al*., 2017)

$$\text{Cfu/ml} = \frac{\text{number of colony x dilution factor } (10^2)}{\text{Volume plated (0.1ml)}}$$

Determination of Microalgae Concentration

The set-ups were monitored at an interval of day(s) 0, 4, 7, and 14 using spectrophotometer at a wavelength of 600nm to monitor the turbidity for each day, from the conical flasks containing *Chaetomorpha*and *Nostoc* species as well as the mixed culture with varying concentrations (4%, 40% and 80% respectively), hence optical density was determined. About 5ml of samples was used and the spectrophotometer was set at 600nm. one (1) ml of the blank (sterile un-inoculated media) was transferred into a labelled polystyrene cuvette using sterile technique to blank the spectrophotometer. Each culture was transferred (using a sterile pipette) into labelled cuvette and the optical density reading was recorded from the spectrophotometer. The optical densities of the controls were determined.

Physicochemical Parameters of the Samples

The physicochemical parameters were determined using standard analytical procedures (AOAC, 2000). The pH meter used was pocket-sized HANA pHep + HI 98108 with automatic temperature compensation. Total organic carbon was determined by dichromate wet oxidation method of Walkley and Black as modified by(AOAC, 2000). Nitrate content was determined using the method of Williams and Dilosi (2018) and available phosphorus was determined using the method reported by(AOAC, 2000). Sulphate was determined using the turbid metric method. Standard methods were used for the determination of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (AOAC, 2000).

Statistical Analysis

The analysis of variance as described by Williams and Dilosi (2018) was used to ascertain the significant difference at 95% confidence interval between the Cyanobacteria total viable counts of the different samples.

III. Results

Cyanobacteria counts

The results of the Cyanobacteriacounts from all the bioremediation set-ups from day 4 to 14 monitored are presented on figures 1 to 3 respectively; Figure 1 shows the changes in the microbial counts in all the samples from 4%. Figure 2 shows the changes in the microbial counts in all the samples from 40%. Figure 3 shows the changes in the microbial counts in all the samples from 80%, for the two species of the microalgae and consortium including the controls.

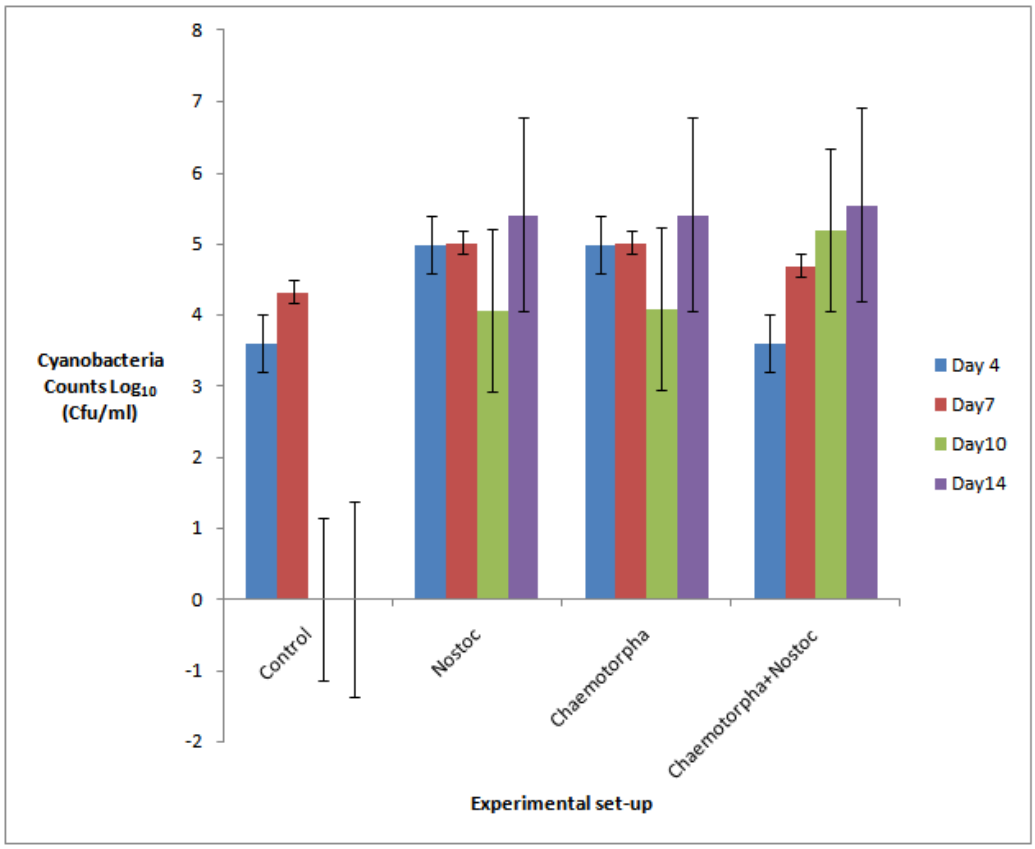

Figure 1: Total Cyanobacteria Counts Log_{10} Cfu/ml from day 4 to 14 from 4% crude oil concentration

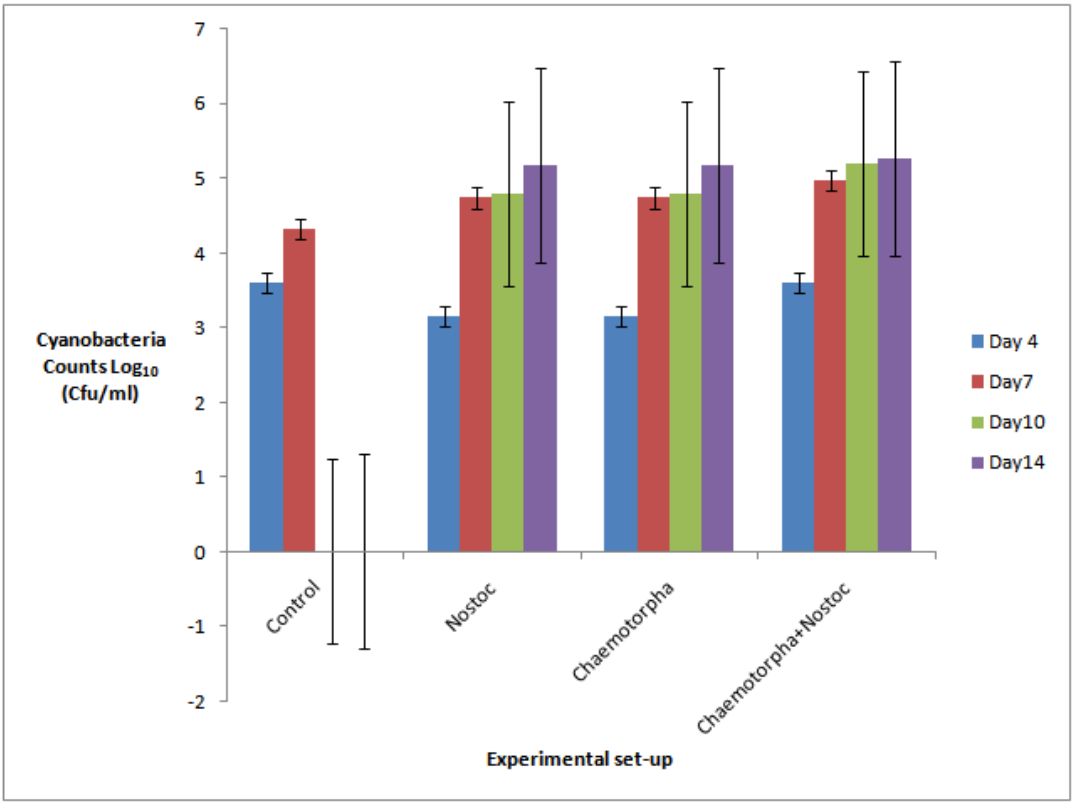

Figure 2: Total Cyanobacteria Counts log_{10} Cfu/ml from day 4 to 14 from 40% crude oil concentration.

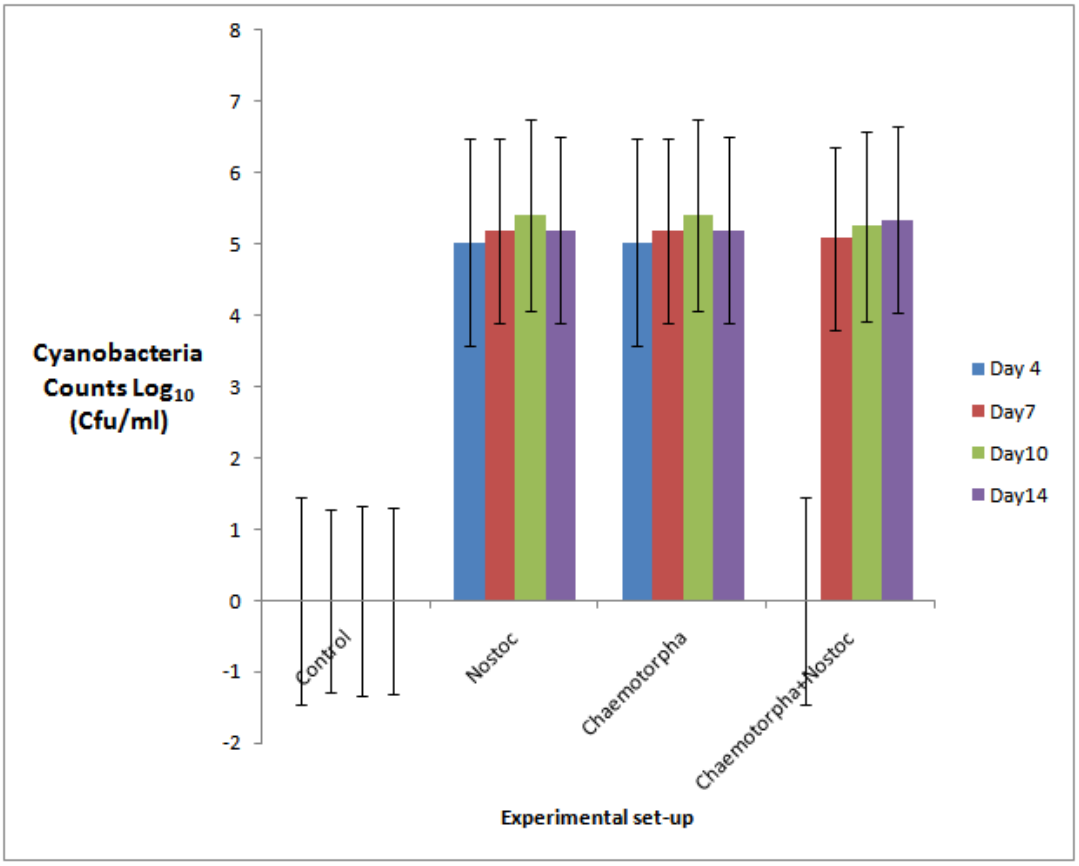

Figure 3: Total Cyanobacteria Counts $\log_{10}$Cfu/ml from day 4 to 14 from 80% crude oil concentration.

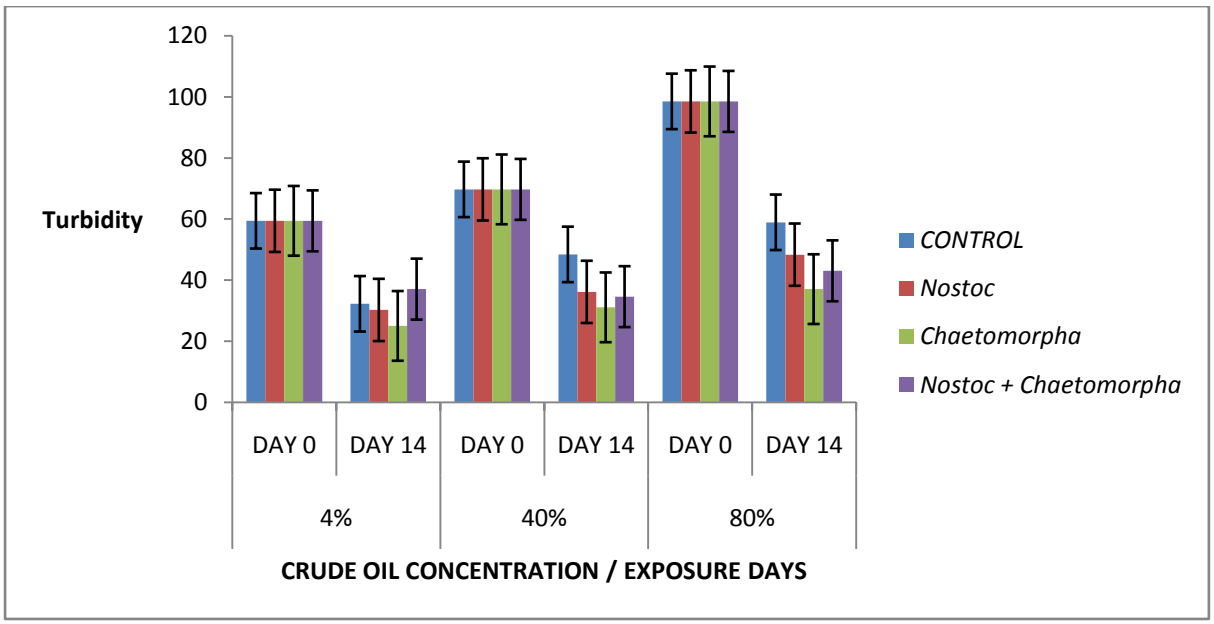

Figure 4: Changes in the Optical Density of the experimental set-ups from the various concentrations of crude oil from day 0 to 14

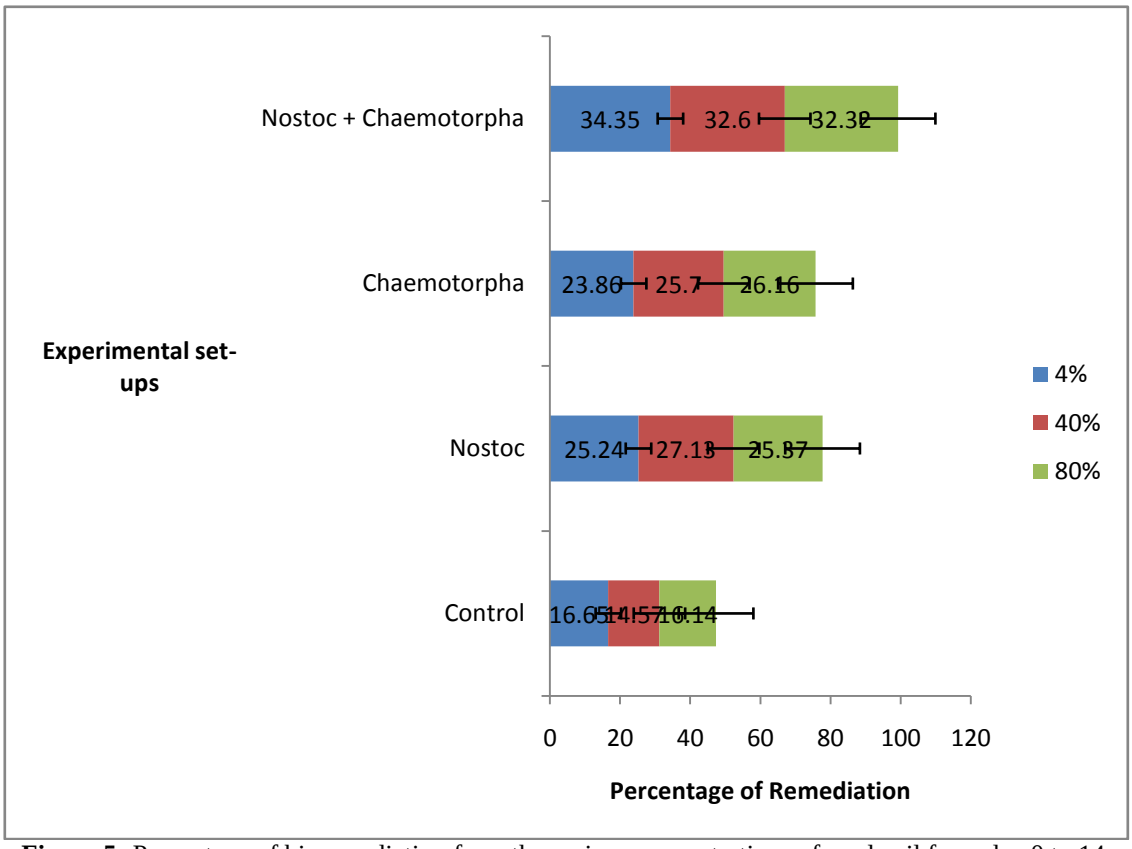

Figure 5: Percentage of bioremediation from the various concentrations of crude oil from day 0 to 14.

Table 1 : Physicochemical parameters analyses for polluted water

S/N	Parameters	Results
1	pH	**6.5**
2	Temperature (^{0}C)	**29**
3	Total organic carbon (mg/l)	**9.9**
4	Nitrogen (mg/l)	**0.14**
5	Phosphorus (mg/l)	**0.78**
6	Sulphate (mg/l)	**6.9**
7	Dissolved Oxygen (mg/l)	**4.5**
8	Biological Oxygen Demand (mg/l)	**2.9**
9	Chemical Oxygen Demand (mg/l)	**4.54**
10	Total Hydrocarbon Content (mg/l)	**35.16**

IV. Discussion

Total Cyanobacterialcounts from all the bioremediation set-ups from day 4 to 14 were monitored as presented on figures 1 to 3 for all the samples; *Chaetomorpha* (OS4%,OS40%, OS80%), *Nostoc* (N4%, N40%, N80%), *Chaetomorpha* and Nostoc(ON14%, ON240%, ON380%).

There was a progressive increase in the total Cyanobacteriacounts from all the set-ups from day 0 to day 14. Fig 5 showed that the consortium set-up had the highest cyanobacteria counts followed by *Chaetomorpha,Nostoc and* Control set-ups. The optical density monitored in this study showed Fig 4 revealed that there was steady increase in the optical densities of the crude oil polluted water samples in all the samples; *Chaetormopha* (OS4%,OS40%, OS80%), *Nostoc* (N4%, N40%, N80%), *Chaetomorpha* and *Nostoc* (ON14%, ON240%, ON380%).The mixed culture (ON) had the highest absorbent rate due to presence of chlorophyll, nutrients (Nitrogen and Phosphorus) and other parameters. There was remediated especially because the increase in optical density grew closer to the optical density of water (1.33) (Williams and Agunkwo,2018). One can deduce that with extension of time, remediation is likely to continue until complete remediation occurs.

The physicochemical parameters showed in table1,revealed that pH and Temperature supported the growth of the test organisms (*Chaetomorpha* and *Nostoc* species) (AOAC, 2000). The nutrients which include Phosphate (0.070–0.072mg/I), Sulphate (1.0–1.2mg/I), and Nitrate (5.1–5.6mg/I) also enhanced the growth of

the organisms (AOAC, 2000). Table 2 showed increase in nutrients such as nitrates (5.7, 5.9 and 6.0mg/l in 4%, 40% and 80% crude oil polluted water, respectively), sulphate (1.8, 2.4 and 2.8 in 4%, 40% and 80% crude oil polluted water, respectively), and phosphate (0.080, 0.90 and 0.98 in 4%, 40% and 80% crude oil polluted water, respectively). This increase occurred as a result of the introduction of the pollutant (crude oil). It was observed that the higher the concentration of pollutant, the greater the increase in the concentration of nutrients. There is no doubt that decrease in dissolved oxygen was obtained due to increase in total organic carbon (TOC) content and other nutrients.

The microbiological analysis showed that there was significant difference in the total Cyanobacteria counts (figs. 2 and 3) which were higher in the mixed culture (ON3 80%) especially on day 7, the last day of analysis. The high counts could be as a result of the presence of a consortium of organisms (ON1, ON2 and ON3) or nutrients (Nitrogen and Phosphorus) that favored the Cyanobacteria growth (Williams and Youngtor, 2017). The analysis of variance (ANOVA) of the total Cyanobacteria count in the samples showed that 80% mixed culture had the highest colonies count. The effectiveness of the cyanobacteria used in this analysis were in the following descending order: 80%ON3> 40%ON2> 4%ON1, 80%O3> 80%N3> 40%O2> 40%N2> 4%O1 4%N1. From the analysis of variance, it showed that 80% mixed culture was more effective and capable of remediating the crude oil polluted water body (Williams and Youngtor, 2017)

Percentage of remediation ranged from 4%; control 16.56<*Chaetomorpha 23.86*, *Nostoc* 25.24 < and *Nostoc+ Chaetomorpha* 34.35. 40%; control 14.57<*Chaetomorpha 25.70Nostoc* 27.13 < and *Nostoc+ Chaetomorpha* 32.60, 80% control 16.14<*Chaetomorpha 25.37*, *Nostoc* 26.16 < and *Nostoc+ Chaetomorpha* 32.32 which shows that set-up with combined cyanobacteria species had the highest remediation percentage. This may be due to synergetic effect of organisms. The control set-ups in all the crude oil concentrations had the lowest remediation percentage which revealed that all the isolates have the capacity to degrade crude oil either as individual or in consortium (Williams and Agunkwo, 2018).

V. Conclusion

The investigation from this study showed that Cyanobacteria species (*Chaetomorpha* and *Nostoc* species) are useful bioremediation tools in crude oil polluted sites. Employment of this method in large scale bioremediation of crude oil pollution is essential. Since there is significant difference among the samples of the Cyanobacteria in their bioremediation percentage, it means that their effectiveness and remediation potential varied in remediating the environment. This work is also coherent with previous studies that showed abattoir effluent and aquaculture water as sources of essential nutrients and media for the cultivation of Cyanobacteria.

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