Bioremediation of Petroleum Products Impacted Freshwater using Locally Available Algae

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Abstract

Bioremediation seeks to degrade or decompose toxic pollutants in the environment into less harmful ones using organisms. This is achieved when the organisms metabolize the pollutants for cellular growth. Algae grow naturally in puddles, drainages and on wet soils and could constitute a nuisance when they cause accidents. This work attempts to investigate the possibility of using naturally occurring algae to reduce hydrocarbon counts in waters contaminated with petroleum products such as petrol, kerosene, diesel and sump oil. Algae were collected from a puddle near Nsukka Fire Service Station. Aliquots of 2 L of tap water with algae properly dispersed were poured into 39 transparent plastic containers and placed in a screened house. Various concentrations of 2.5 ml/L, 5 ml/L and 7.5 ml/L of pollutants were introduced in three replicates. A control was set up without these. Physico-chemical (pH, dissolved oxygen content, nitrates, phosphates, ammonia, copper, zinc, iron and magnesium) and phycological analysis were carried out using standard methods at the beginning and end of the experiment that lasted for 40 days. Cyanobacteria (Oscillatoria) and Chlorophyta (Spirogyra) were dominant at the onset. Later, more growth of Chlorophyta (Clostrium, Spirogyra, Ulothrix and Ankistrodesmus) and Cyanobacteria, (Aphanizomenon, Oscillatoria, Microcystis) were observed, while Bacillariophyta (Navicula and Pinnularia) and Dinophyta (Peridinium) were low. Results showed a drop in temperature, pH, oxygen percentage saturation, total alkalinity and hydrocarbon and increase in nitrate nitrogen, phosphate, magnesium, iron and zinc with time.

Keywords: Bioremediation, Algae, Chlorophyta, Cyanobacteria, Petroleum products

Introduction

The problem of oil pollution in Nigeria is enormous. The pollution of the environment by petroleum products is an inevitable manifestation of oil production, transportation, and distribution activities and political instability. Such spills could be catastrophic to the environment. By nature of its composition, petroleum is a heterogeneous and water insoluble substrate that presents special problems to organisms found in the freshwater environment into which it is spilled.

Biodegradation is a natural process whereby organisms can alter and breakdown wastes, especially petroleum hydrocarbons into other substances. The resulting products can be carbon dioxide, water and partially oxidized biologically inert by-products (Al Hassan *et al.,* 1994). Biodegradation is a cheap technique for cleaning out both organic and inorganic wastes from the environment.

Hydrocarbon degrading microbes (bacteria, fungi and algae) are widely distributed in marine, fresh water and soil habitats whether polluted or not. This distribution of microbes is of tremendous importance in view of their use in the treatment of oil spills (Amund, 2000). These microbes evolve diverse biochemical processes to breakdown chemical constituents and metabolize petroleum and algae decompose straight chains hydrocarbons readily (Onwurah, 2000; Cohen, 2002).

All algae are dependent on nutrients for survival. The nutrients are the basic building blocks of life and allow them create the necessary enzymes to break down hydrocarbons (Lee, 1992; Cohen, 2002). The basic nutrients are carbon, nitrogen, phosphorous and some other macro elements such as magnesium, zinc and copper. These with some physico-chemical factors are responsible for the distribution and growth of algae. Such factors include dissolved oxygen, temperature, colour, pH, alkalinity and other dissolved ions (Hutchinson, 1975; Harris, 1986).

Some algae have been utilized in the process of bioremediation. In Ohio State University, strains of *Chlamydomonas reinhardtii* have been utilized to clean toxic waste water. This alga has been implicated in the removal of mercury, lead, copper and other metals from contaminated water (Uno *et al*., 2001). Al-Hassan *et al*. (1994) showed that mats of Cyanobacteria were found on water contaminated with petroleum and they successfully helped in cleaning up the coasts of the gulf of Iran. Nwankwo (2000) showed also that they were found on fresh crude oil impacted soil in the Niger-Delta area of Nigeria, only one month after impact. The predominant algae were the Chlorophyta, Cyanophyta and Euglenophyta.

Petroleum on leakage is subject to a series of diverse physical and chemical changes that are dependent on the nature of the oil and the environmental conditions. Volatile petroleum products such as petrol upon spillage evaporate readily followed by diesel and kerosene, while crude oil and sump oil form oily mats on the water surface.

Algae are readily available and grow naturally in puddles, drainages and on wet soils as first colonisers; and could constitute a nuisance when they cause accidents.

As noted by Onwurah (2000) environmental pollution control biotechnology encompasses prevention, control and remediation of environmental pollution, using biotechnological base products such as microorganism, enzymes and other relevant engineering processes. This work attempts to investigate the possibility of using locally available algae to reduce hydrocarbon counts in waters contaminated with some petroleum products - petrol, kerosene, diesel and sump oil.

Materials and Methods

Petrol, kerosene and diesel were purchased at an Oando filling station in Nsukka. Sump oil was collected from car engines serviced in a car service centre at Nsukka. The algae used for the investigation were collected from mud along the University road, near Nsukka Fire Service Station. Tap water was collected from a University of Nigeria borehole and poured into a 150 L container, seeded with the mud laden algae and properly dispersed by stirring vigorously with a stick. Aliquots of 2 L of this water were poured into the 39 transparent plastic containers, covered with plastic mosquito netting and placed in a screened house at the University of Nigeria, Botanical Garden.

Phycological studies were carried out on the contaminated water to determine the composition of algae and at the onset and the end of the experiment. About 20 ml of sample was fixed with Lugol's iodine prepared following Bellinger (1992). Algae were viewed under a Leica binocular Microscope at X 400 magnification and identified following the methods of Prescott (1962) and Bellinger (1992).

Petroleum products such as petrol, kerosene and diesel, and sump oil were introduced into the water at various concentrations of 2.5 ml/L, 5 ml/L, and 7.5 ml/L and replicated in threes. A control experiment was set up without these products. The treatments and the control were left for 40 days following the observations of Nwankwo (2000) that algae are able to survive stressed conditions such as crude impacted soil one month after impact.

Physico-chemical parameters of the contaminated water sample were analysed at the Public Health Laboratory of the Department of Civil Engineering and in the Department of Botany, both at the University of Nigeria; and also in the Analytical Laboratory of Aluminum Smelter Company of Nigeria (Alscon) Ikot-Abasi, Akwa-Ibom State following the methods of APHA (1995). Parameters analysed include temperature, dissolved oxygen content, alkalinity, pH, total hydrocarbon content, Nitrate, phosphate, ammonia, sulphide; heavy metals such as copper, magnesium, zinc and iron.

Temperature (^{0}C) was determined by using a thermometer; dissolved oxygen by Azide modification of Winkler titration method; total alkalinity (mg/L $CaCO_3$), titrimetrically with N/50 H_2SO_4 and methyl orange indicator; pH with a digital pH meter (Hanna instruments) and Nitrate, phosphate, ammonia and sulphides, colorimetrically using a Lovibond comparator and subsequently spectrophotometrically using Spectronic – 20. Magnesium, copper, and zinc were determined using an Atomic Absorption Spectrophotometer while total hydrocarbons and iron were carried out using a Hach 2000 DR spectrophotometer.
Phycological data are presented as percentage composition.

Results

Physico-chemical factors: The initial water temperature was 30 ^{0}C. After treatment with various concentrations of petroleum products, there was a drop in water temperature at all concentrations. Final temperatures ranged from 23.8^0 –28 ^{0}C (Fig.1). The pH of the initial sample was slightly acidic (6.6), so also were the samples after treatment (5.85 – 6.65). There was a drop in the pH of the samples at the end of the experiment with samples treated with Diesel having the least values as shown in Fig. 2.

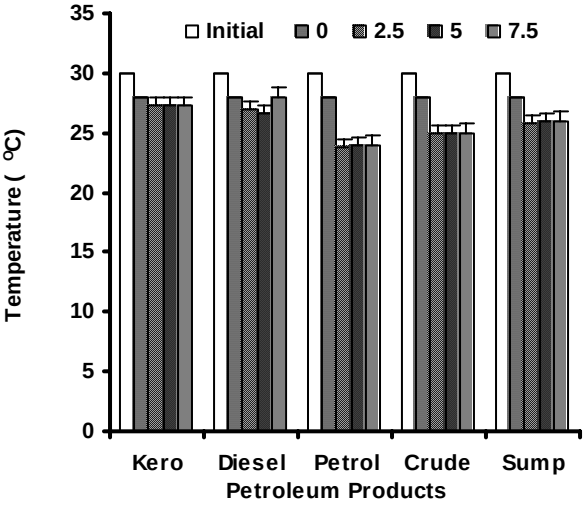

Fig. 1: Variations in temperature of samples treated with various petroleum products

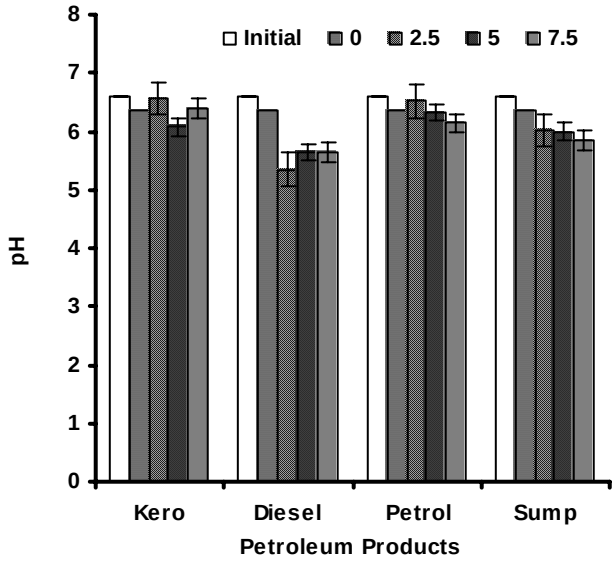

Fig. 2: Variations in pH of samples treated withvarious petroleum products

Initial oxygen concentration of algae seeded water was high (9 mg/L) and supersaturated (121.3%). There was a significant drop in the dissolved oxygen content after contamination with petroleum products to lower levels (range, 1.9 mg/l – 6.66 mg/l) with sump oil having the least value (Fig. 3).

There was a decline in alkalinity after treatment with petroleum products (Fig 4).The total hydrocarbon content decreased for all the petroleum products at the end of the experiment (Table 1). Nitrate nitrogen in the treated samples was lower than initial samples for all petroleum fractions (Table 1).

Phosphate levels ranged from 0.3 - 2.68mg/l. Final phosphate concentrations were higher than those at the beginning of the experiment for all petroleum products, except for sump oil with lower concentrations for higher contamination (Table 1). Ammonia concentration did not show any particular trend, but in the treatment with kerosene at 7 ml/L of water, final ammonia was lower while the converse was the case for diesel and petrol (Table 1).

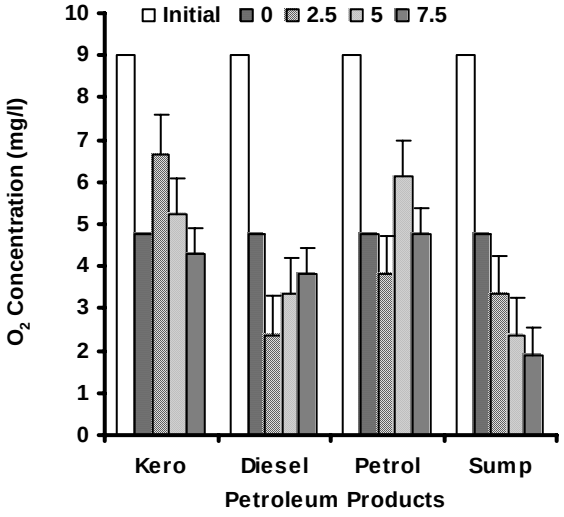

Fig 3: Variations in oxygen concentrations of samples treated with various petroleum products

At lower concentrations, final sulphide was lower than initial values for kerosene; for diesel final concentrations were lower for all dilutions; for petrol there were no variations for the dilutions and for sump oil the initial values were higher at lower concentrations (Table 1).

Final concentrations of copper were lower for kerosene at 2.5 ml/L contamination, and higher for high concentration (7.5 ml/L) for all petroleum products (Table 1). Final Magnesium and iron concentrations after treatment were higher than the initial for all petroleum products respectively. Final zinc concentrations after treatment were lower initial concentration for all petroleum products (Table 1).

Phycological results: The percentage algal composition of the control experiment and various treatments at the onset and end of the experiment are presented in Table 2.

Initial percentage composition of algae in the control showed that Cyanobacteria were highest (61.43%) followed by Chlorophyta (34.62%) and Bacillariophyta (3.94%) in decreasing order. At the end of the experiment, the trend was the same but Bacillariophyta and Dinophyta were not observed.

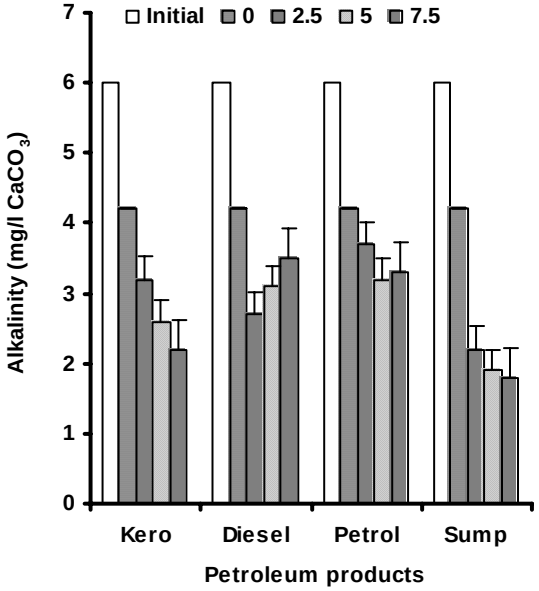

Fig 4: Variations in alkalinity of samples treated with various petroleum products

Final Cyanophyta was highest in the experiments with kerosene, diesel and petrol at 2.5 ml/L (73.28 %; 59.22 % and 52.38 % respectively) and 5 ml/L (72.16 %; 94.34 % and 53 % respectively). They were low at high contaminant level (7.5 ml/L) and in all dilutions of sump oil while Chlorophyta were highest in treatments with kerosene and diesel at 7.5 ml/L (67.37 % and 60 % respectively) and all treatments with sump oil, 2.5 ml/L (50.59%); 5 ml/L (51.1%) and 7.5 ml/L (65.71 %). Percentage composition in treatment with 7.5 ml/L petrol showed equal percentage composition of both groups. Bacillariophyta and Dinophyta were low throughout the experiments.

Final percentage population of Cyanobacteria in all treatments with 7. 5 ml petroleum product per litre of water was lower (13.6, 40, 50 and 34.29% for kerosene, diesel, petrol and sump oil respectively). On the other hand, final percentage saturation of Chlorophyta was highest at this dilution for all products (87.37, 60, 50 and 65.71% for kerosene, diesel, petrol and sump oil respectively).

Discussion

There was a drop in temperature, pH, oxygen, total alkalinity and hydrocarbon counts at the end of the experiment.

Table 1: Variations in total hydrocarbon, nitrate, phosphate, ammonia, sulphide, copper, magnesium, iron and zinc contents in various freshwater samples impacted with kerosene, diesel, petrol and sump oil concentrations

Concentrations	Kerosene		Diesel		Petrol		Sump oil	
	initial	final	initial	final	Initial	final	initial	Final
Total Hydrocarbon								
0	2.98	1.40	2.98	1.40	2.98	1.40	2.98	1.40
2.5	60.60	6.08	17.23	10.67	132.63	29.64	285.6	98.36
5	120.13	39.69	34.45	18.59	265.26	34.82	570.99	98.85
7.5	179.90	14.39	50.98	29.94	397.00	85.98	782.86	14.147
Nitrate								
0	0.20	0.50	0.20	0.50	0.20	0.50	0.20	0.50
2.5	1.70	3.20	0.90	2.00	0.80	2.30	1.40	3.00
5	1.30	2.10	1.30	2.40	0.90	2.10	1.00	2.00
7.5	0.90	1.40	1.10	2.30	0.60	1.80	1.20	2.40
Phosphate								
0	2.52	2.68	2.52	2.68	2.52	2.68	2.52	2.68
2.5	2.52	2.68	1.60	1.87	2.20	2.68	1.30	1.88
5	1.60	2.00	1.20	1.34	2.20	2.68	1.27	1.07
7.5	1.52	1.87	1.20	1.68	2.45	2.68	1.20	1.07
Ammonia								
0	0.008	0.011	0.008	0.011	0.008	0.011	0.008	0.011
2.5	0.010	0.01	0.052	0.026	0.006	0.012	0.006	0.006
5	0.014	0.014	0.056	0.056	0.056	0.01	0.008	0.010
7.5	0.028	0.010	0.008	0.044	0.010	0.06	0.012	0.014
Sulphide								
0	4.00	2.00	4.00	2.00	4.00	2.00	4.00	2.00
2.5	6.00	2.00	4.00	2.00	2.00	2.00	4.00	2.00
5	4.00	4.00	4.00	2.00	4.00	4.00	4.00	2.00
7.5	4.00	4.00	4.00	2.00	3.00	3.00	2.00	2.00
Copper								
0	0.001	0.012	0.001	0.012	0.001	0.012	0.001	0.012
2.5	0.008	0.024	0.001	0.001	0.001	0.001	0.120	0.079
5	0.015	0.018	0.003	0.024	0.003	0.009	0.03	0.018
7.5	0.0229	0.010	0.005	0.084	0.005	0.022	0.006	0.053
Magnesium								
0	1.781	30.00	1.781	30.00	1.78	30.00	1.781	30.00
2.5	2.371	14.50	1.349	9.59	3.50	21.20	1.424	11.60
5	1.83	20.30	1.350	6.70	1.88	24.30	1.657	11.60
7.5	2.008	4.70	1.823	8.06	2.12	8.60	1.363	18.30
Iron								
0	0.018	0.026	0.018	0.026	0.018	0.026	0.018	0.026
2.5	0.002	0.009	0.008	0.009	0.012	0.116	0.004	0.101
5	0.002	0.063	0.036	3.12	0.012	0.221	0.008	1.831
7.5	0.002	0.080	0.90	2.06	0.008	0.082	0.008	0.01
zinc								
0	0.087	0.03	0.087	0.03	0.087	0.03	0.087	0.03
2.5	0.215	0.035	0.34	0.054	0.252	0.011	2.25	0.422
5	0.43	0.29	0.675	0.14	0.54	0.061	4.505	0.742
7.5	0.64	0.37	0.90	0.163	0.70	0.378	6.50	0.637

Table 2: Percentage algal composition of control experiments and various treatments with kerosene, diesel, petrol and sump oil

Algae	Percentage Composition													
	Control		Kerosene (ml/L)			Diesel (ml/L)			Petrol (ml/L)			Sump oil (ml/L)		
	Initial	Final	2.5	5	7.5	2.5	5.0.00	7.5	2.5	5.0.00	7.5	2.5	5	7.5
Cyanobacteria (Blue-green algae)														
Aphanizomenon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.52	0.00
Microcystis	0.00	0.00	0.00	0.00	0.00	16.92	28.20	20.00	0.00	0.00	0.00	0.00	0.00	0.00
Oscillatoria	61.43	65.10	73.28	72.16	13.63	42.30	56.00	20.00	52.38	53.3	50.00	46.88	16.67	34.29
Total	61.43	65.10	73.28	72.16	13.63	59.22	84.20	40.00	52.38	53.3	50.00	46.88	35.19	34.29
Chlorophyta (Green algae)														
Ankistrodesmus	3.77	0.00.83	0.00	0.00	0.00	17.69	0.00	40.00	0.00	0.00	50.00	0.00	45.30	57.14
Spirogyra	30.85	35.07	0.86	0.00	64.64	23.08	20.44	10.00	0.00	33.3	0.00	50.59	10.25	8.57
Ulothrix	0.00	0.00	25.86	26.29	22.73	0.00	0.00	10.00	28.57	0.00	0.00	0.00	0.00	0.00
Closterium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.4	0.00	0.00	0.00	0.00
Total	34.62	35.90	26.72	26.29	87.37	40.77	20.44	60.00	28.57	46.7	50.00	50.59	55.55	65.71
Bacillariophyta (Diatoms)														
Fragillaria	0.00	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.00	9.26	0.00
Navicula	1.97	0.00	0.00	1.55	0.00	0.00	0.00	0.00	19.0.004	0.00	0.00	0.00	0.00	0.00
Pinnularia	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	3.94	0.00	0.00	1.55	0.00	0.00	5.56	0.00	19.0.004	0.00	0.00	0.00	9.26	0.00
Dinophyta														
Peridinium	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.52	0.00	0.00
Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.52	0.00	0.00

Furthermore, the levels of nitrate nitrogen, phosphate, magnesium, iron and zinc increased at the end of the experiment. The drop in temperature could be associated with the cooling effect of the petroleum products in the case of kerosene, petrol and diesel; and the period of study which is characteristically lower because of the prevailing rain clouds. Before contamination with petroleum products the water was supersaturated with oxygen. This was lowered by the contaminants which prevented further diffusion from the atmosphere and caused more respiratory stress to the algae and other organisms. The decrease in the levels of oxygen, from 9.0 mg/l (super saturated level) in the control at the beginning of the experiment to lower levels (under saturated, range, 1.78 mg/l – 6.66 mg/l) was as a result of the addition of the contaminants. The algae that adjusted were able to grow under oxygen stress. Higher phosphate content at the end of the experiment was related to its utilization in the build-up of algal biomass and other cellular activities (Chapman and Chapman, 1977; Lee, 1992). Also, it must have been elevated by eutrophication of the water caused decomposition processes that released nutrients. Lower final phosphate in sump oil is associated with the shading effect of the oil that reduced the penetration of light hence lower algal productivity.

Introduction of contaminants into the water also led to drastic reduction in alkalinity from 6.0 to 1.8 mg/l $CaCO_3$. There was a decrease in the concentration of total hydrocarbons in the water sample from very high levels to lower levels as a result of the activity of algae and in conjunction with other microbes as noted by Amund (2000). Also, increased nitrates during the experiment can be attributed to breakdown of the crude oil and the other petroleum products by algae and bacteria and its use in the build - up of biomass by these organisms.

The drop in the total hydrocarbon content in the contaminated water from the beginning of the experiment to lower levels at the end of the experiment indicate that there was breakdown of the hydrocarbons. Algae and bacteria are implicated in their breakdown (Amund, 2000; Nwankwo, 2000).

The drop in pH of contaminated samples indicates that these petroleum products increase acidity, which can induce changes in biodiversity of contaminated environments. The increase in magnesium after treatment was as a result of algal growth and associated increase in chlorophyll content of which magnesium is an important component (Uno et al., 2001). Also, the increase in the concentration zinc at the end of the experiment is associated with algal metabolism of the element from the contaminants, zinc, being an important micronutrient for plant metabolism (Lee, 1992).

Phycological analysis showed that at the onset of the experiment 5 taxa of algae belonging to phylum Cyanobacteria (*Oscillatoria*), Chlorophyta (*Akistrodesmus* and *Spirogyra*) and Bacillariophyta (*Navicula* and *Pinnularia*) were observed. Two phyla were predominant - Cyanobacteria (63.4%) with *Oscillatoria* dominating and Chlorophyta (36.62%) with *Spirogyra* dominating (30.85%). Bacillariophyta were low (3.94%). By the end of the experiment Dinophyta represented by *Peridinium* was observed, but few in number. Invasive species such as A*phanizomenon Microcystis*, *Ulothrix, Closterium, Fragillaria and Peridinium* that were encountered later were not sustained. Only *Oscillatoria* (Cyanobacteria); *Spirogyra* and (Chlorophyta) maintained their presence in most dilutions of the petroleum products. These facts are in line with the findings of Alhassan *et al.* (1994) in the Gulf of Iran and Nwankwo (2000) in the Niger Delta area of Nigeria that Cyanobacteria and Chlorophyta are potential bioremediation and biodegradation tools.

There is no doubt that the petroleum products are stressors in the environment. There is evidence that the dominant groups – Chlorophyta and Cyanobacteria survive in a stressed or polluted environment while the other classes could not survive affirming the opinions of Round, (1973) and Chapman and Chapman (1977) that the eliminated taxa (*Navicula, Pinularia* and *Peridinium*) thrive better in clear flowing water. The algae that thrived at the end of the experiment could be considered as adaptive strains that could be useful for further studies.

Sump oil is of great nuisance and has high ecological risks because of its high hydrocarbon count. Results from this study suggest that Chlorophyta were more tolerant at higher concentrations suggesting their suitability in bioremediation of petroleum oil products (especially sump oil) impacted freshwaters and soils. Prominent genera were *Spirogyra* and *Ulothrix*. These taxa have been reported in various locations in Nigeria (Biswas and Nweze, 1990; Opute, 1999; Kadiri, 1999; Nweze and Domrufus, 2006). Further work using isolated species of these taxa is recommended for further research in environmental pollution control biotechnology.

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