academicJournals

Vol. 8(9), pp. 375-381, 4 March, 2013
DOI 10.5897/SRE12.730
ISSN 1992-2248 © 2013 Academic Journals
http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Determination of the relative abundance and distribution of bacteria and fungi in Bonny light crude oil-contaminated sandy loam soil

Eze C. N.*, Ogbonna J. C., Anyanwu C. U. and Eze E. A.

[1]Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Accepted 21 February, 2013

This study was undertaken to investigate the effects of Bonny light crude oil (specific gravity = 0.81; API gravity 43.2^0) on the numerical composition of soil bacteria and fungi and relative distribution of Gram positive and Gram negative soil bacteria. Eight different levels of the crude oil (0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0 or 20.0% v/w of soil) were used for the controlled pollution of soil samples obtained from the Botanical Garden, University of Nigeria Nsukka. Studies on the effects of crude oil on bacterial and fungal populations were carried out by plate count procedures using nutrient agar and Sabouraud dextrose agar respectively. Crude oil significantly ($p<0.05$) inhibited bacterial and fungal growth in a dose-dependent manner. Bacterial counts in the control soil sample ranged between 2.32×10^9 and 2.80×10^9 cfu/g while those of the contaminated samples ranged between 2.00×10^8 and 2.77×10^9 cfu/g. Fungal counts ranged from 1.02×10^7 to 1.39×10^7 cfu/g in the control and 1.60×10^5 to 1.18×10^7 cfu/g in the contaminated samples. At 15.0 and 20.0% levels of crude oil, the growth inhibitory effects of crude oil were maximum for bacteria and fungi respectively. Microbial respiration decreased concomitantly with increase in crude oil pollution during the first four weeks of the study. There was a prevalence of Gram positive bacteria over Gram negatives in the unpolluted soil but a preponderance of Gram negative rods over Gram positives and other morphological forms of Gram negatives in the polluted samples.

Key words: Bonny light crude oil, bacteria, fungi, contamination, hydrocarbon, biodegradation.

INTRODUCTION

The discovery of petroleum brought significant transformation on the planet earth. It changed the pace of civilization through mechanization and industrialization. In spite of these positive changes attributable to petroleum, it also ushered in its wake environmental pollution causing untold havoc to the biotic and abiotic components of the ecosystem (Millioti et al., 2009). According to Onuoha et al. (2003), when crude oil comes in contact with the soil it results in damage to agricultural lands, microorganisms and plants. Crude oil and its products are made up of hydrocarbons which modify the physical and chemical properties of soil and its structure (Chi Yuan and Krishnamurthy, 2000; Terrence et al., 2011). Soil polluted by petroleum loses its activity and may take up to ten years to recover (Sparrow and Sparrow, 1988; Racine, 1993; Wyszkowska et al., 2001).

Owing to the lipophilicity and hydrophobicity of crude oil, it prevents both air and water exchange between soil and atmosphere (Atlas, 1977). Apart from its indirect effect through modification of the soil environment, smearing soil organisms with oily substances can reduce their cell membrane permeability lowering or entirely

*Corresponding author. E-mail: chibuzoreze@gmail.com.

blocking nutrient absorption which eventually leads to starvation and death (Pezeshki et al., 2000). Crude oil pollution has also been reported to introduce into the environment, non-organic, carcinogenic and growth inhibiting chemicals which are toxic to both microorganisms and man (Atlas and Bartha, 1973a; Odu, 1972, 1978; Okpokwasili and Odokuma, 1990; She et al., 2012).

Microorganisms because of their ubiquity and simple nature are usually the first to report the presence of pollutants in any environment. This occurs through marked numerical or functional changes in their population or genetic changes causing mutation in individual cells. Soil microorganisms play a vital role in the sustenance of the terrestrial ecosystem. They play major roles in the detoxification of wastes such as heavy metals and radionuclides, recycling of elements (for example, carbon, nitrogen, sulphur, phosphorus etc) and clearing up of oil spills (Funke, 1998; Singleton, 1997). This latter role is presently receiving global attention because of the increase in crude oil mining activities coupled with poor maintenance of oil pipelines and transportation vessels. These lead to release of crude oil and its products into the soil and aquatic environments in increasing amounts. Even though some microorganisms have inherent hydrocarbonoclastic potentials (Atlas and Bartha, 1973a), expression of the trait may be undermined by very high levels of crude oil pollutant in the environment which will cause an imbalance in the carbon-nitrogen (C:N) ratio. Such imbalance occurs through oil spills and is deleterious to microorganisms (Adoki and Orugbani, 2007).

Considering the overwhelming role of microorganisms in the soil environment, it is therefore imperative that this component of ecological flora be preserved. Even though there are some blanket reports on the toxicity effects of crude oil on microorganisms, there is a definite lack of research information on its effects on sandy loam soil microorganisms, specifically addressing effects on microbial population, respiration and Gram distribution of the bacterial population. To this end, this study has been undertaken to evaluate the effects of Bonny light crude oil on microorganisms in sandy loam soil with particular focus on the afore-mentioned parameters.

MATERIALS AND METHODS

The major materials used in the study were:

1. Bonny light crude oil which was collected from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt Refinery, Alesa-Eleme, Rivers State, Nigeria.
2. Pristine sandy loam soil collected from Botanical Garden, University of Nigeria, Nsukka.

Determination of the effects of crude oil on soil bacteria and fungi

Pristine sandy loam soil was air-dried, sieved and dispensed in 0.5 kg weights into nine plastic pots (13 cm deep × 12 cm diameter). Each pot, apart from the control, received one of eight different levels of Bonny light crude oil (0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0 or 20.0% v/w). This broad range of crude oil concentrations was used to provide a more reliable means to assess the influence of concentration of oil pollutant on microbial population size in the soil habitat. The soil in each pot was homogenized after crude oil addition to achieve a uniform distribution of the oil, and subsequently watered every four days by spraying. Microbiological analysis to determine microbial counts and Gram distribution of bacteria was carried out with 1.0 g of soil collected from each pot at weekly intervals and diluted ten-folds using sterile normal saline. The population of viable bacterial and fungal cells in each soil sample was determined by inoculating 0.1 ml aliquots from the 10^{-8} dilution onto nutrient agar and sabouraud dextrose agar respectively by the spread plating technique as described by Wistreich (1997). Sabouraud Dextrose agar was further made selective for fungi by the incorporation of 50 µg of chloramphenicol/ml (v/v). Incubation was at 28°C for 24 h and 5 days for bacteria and fungi respectively. Determination of the effect of crude oil on the Gram distribution of soil bacteria was obtained by Gram staining bacterial colonies after incubation and expressing the Gram positive and Gram negative colonies from the polluted and unpolluted soils as percentage.

Determination of the effect of petroleum on soil respiration (Stotzky, 1965)

Procedure

Fifty grams of each soil sample, collected at four-weekly intervals, was weighed in duplicate into beakers placed inside jars with air-tight covers. Twenty-five millilitres of 0.05 M NaOH was pipetted into each jar and immediately the jars were made airtight with rubber rings. Three jars containing 0.05 M NaOH but without soil were used as controls for both polluted and unpolluted soil samples. Thereafter all jars were incubated for 3 days at 25°C (room temperature).

Estimation of CO_2

At the end of the incubation the jars were opened and the beakers taken out. The external surface of each beaker was washed with CO_2-free water (prepared by cooling boiled distilled water in a container with CO_2 absorption tubes) to wash the NaOH solution completely into the jar. Thereafter, 5 ml of 0.5 M barium chloride solution was added to each jar and some drops of phenolphthalein indicator incorporated. Hydrochloric acid (0.05 M) was added drop-wise with continuous stirring until the colour changed from red to colourless.

Calculation of results:

The rate of the respiration was calculated by the following relationship:

$$CO_2 \text{ (mg)} / SW/t = \frac{(Vo - V) \times 1.1}{DWT}$$

Where SW is the amount of soil dry weight in grams, T is the incubation time in hours; Vo is the volume of HCl used for titration (average value) in milliliters.

V is the volume of HCl used for the soil sample (average value), DWT is the dry weight of 1 g moist soil and 1.1 is the conversion factor (1 ml 0.05 M NaOH equals 1.1 mg CO_2).

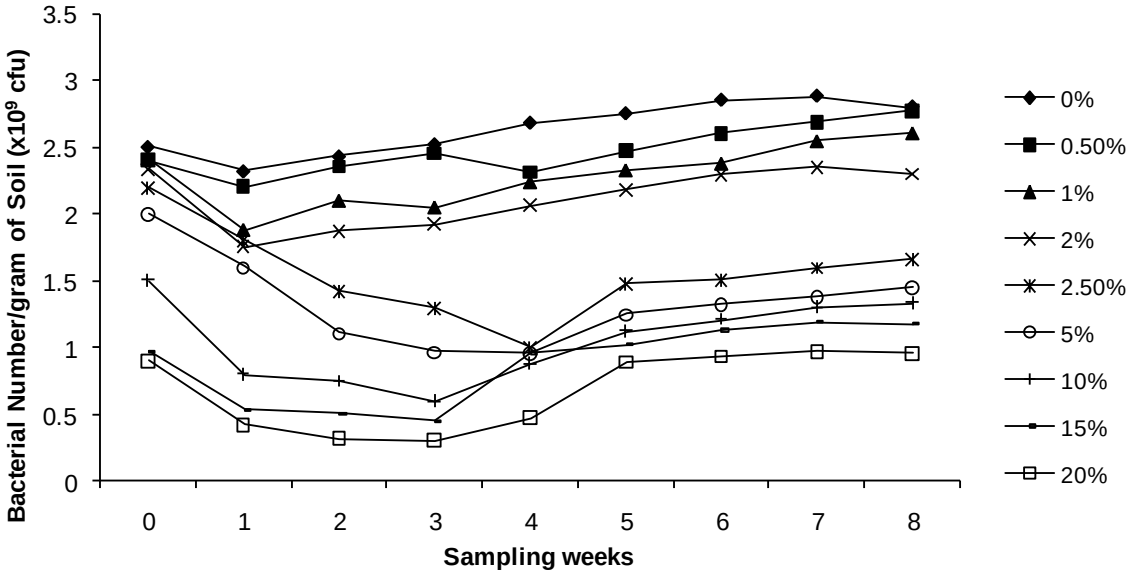

Figure 1. Effects of varying levels of crude oil on total colony count of soil bacteria.

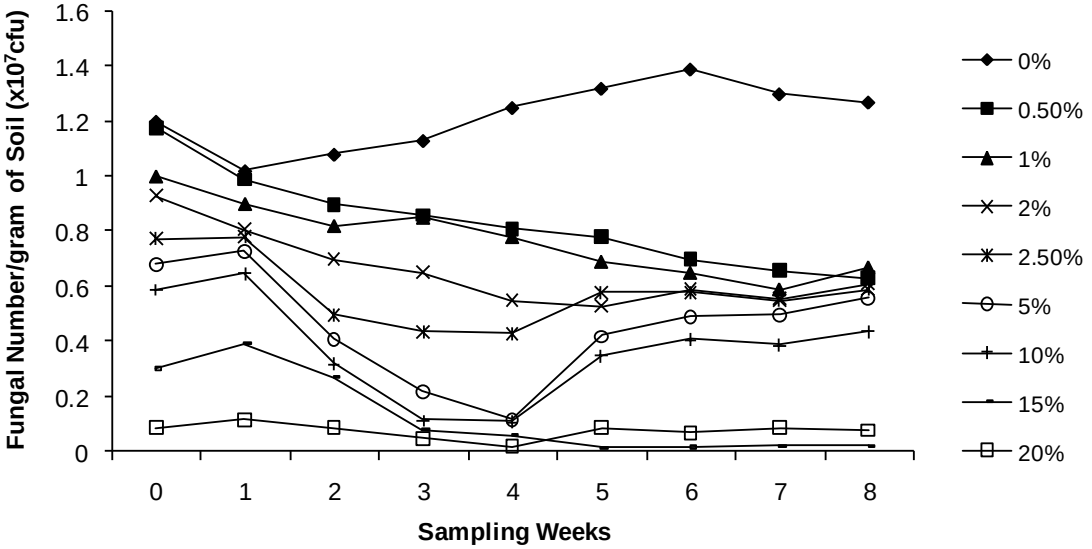

Figure 2. Effects of varying levels of crude oil on total colony count of soil fungi.

RESULTS

Figure 1 depicts the effects of varying levels of crude petroleum on total colony count of soil bacteria. At low concentrations (0.5 to 2.0%) of the crude oil, bacterial numbers were not significantly ($P<0.05\%$) affected. It was only within the first week that a slight decline in number occurred (for concentrations, 0.5 to 2.0%), (Figure 1) after which there was a gradual but steady rise in population till the eight week. On the contrary, crude oil at high concentrations (2.5 to 20%) had a significant ($P<0.05$) negative effect on bacterial numbers. From 2.5% crude oil level there was a sharp decline in bacterial numbers from the first week up to the fourth week after which a gradual rise in population occurred till the eighth week.

The negative effect of crude oil on fungi appeared to be more pronounced than the effects on bacteria. This is indicated by the progressive decline in fungal numbers at all crude oil concentrations (Figure 2). At higher concentrations (2.5 to 20.0%) however, the inhibitory effects of the oil on fungal numbers increased, attaining peak levels at 15 and 20% crude oil contamination.

Figure 3 shows the relative abundance of Gram positive

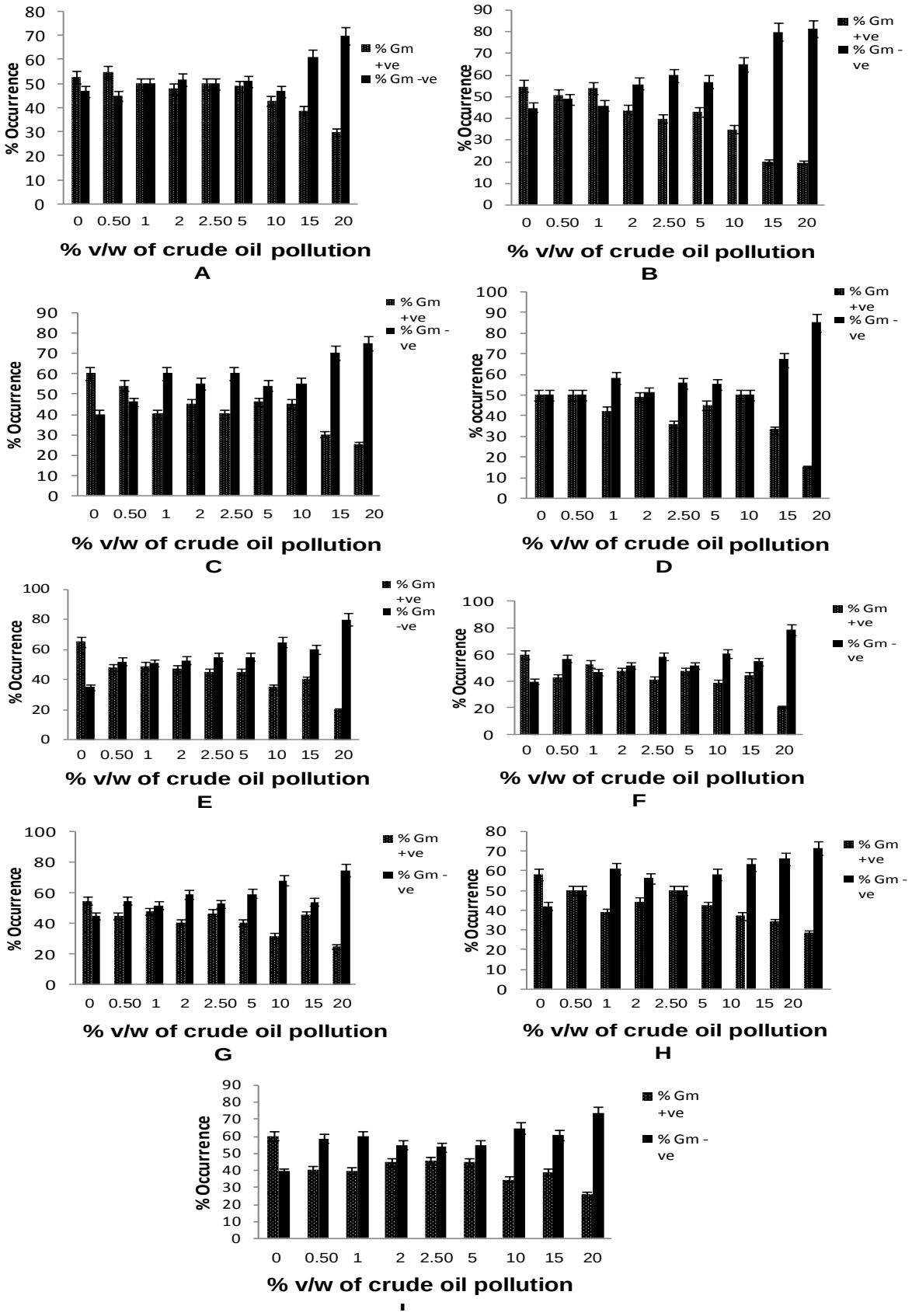

Figure 3(a-i). Relative abundance of Gram positive and Gram negative bacteria in crude oil-polluted and unpolluted soil samples. A=Week 0, B=Week 1, C=Week 2, D=Week 3, E=Week 4, F=Week 5, G=Week 6, H=Week 7, I=Week 8.

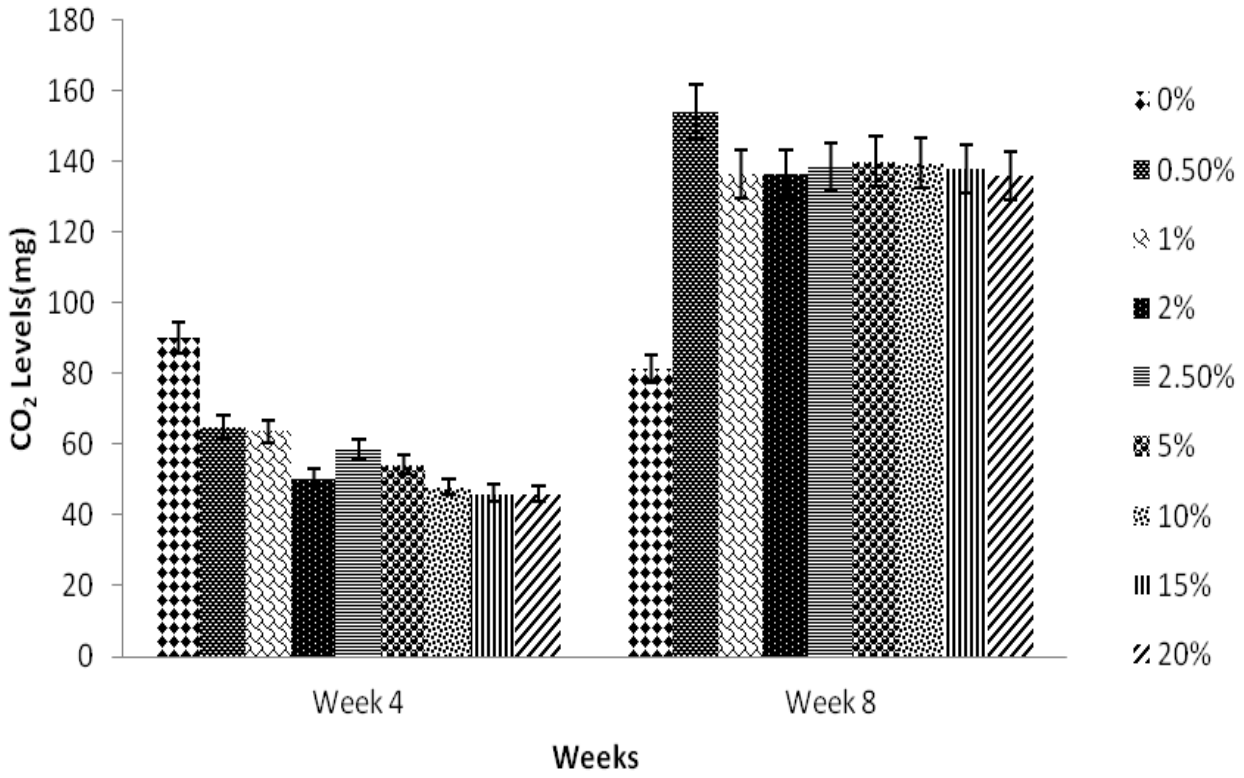

Figure 4. Effects of crude oil on soil respiration.

and Gram negative bacteria in the control and crude oil polluted soils. There was a preponderance of Gram positive bacteria over the Gram negative in the unpolluted controls but in the polluted soils, the populations of Gram negative bacteria, particularly rods predominated over the Gram positive.

Result of the effects of crude oil on soil microbial respiration is presented in Figure 4. Soil respiration was quantified in terms of the level of carbon IV oxide evolved from the soil. Analysis for CO_2 evolution was done at four-weekly intervals during the eight-week experiment. High levels of crude oil significantly ($P<0.05$) reduced the level of CO_2 evolution during the first four weeks of the experiment (Figure 4).

DISCUSSION

At low concentrations of crude oil (0.5 to 2%), bacterial growth was slightly enhanced as indicated by the low increase in bacterial numbers after one week of the experiment (Figure 1) Enhancement of microbial activity at low doses of petroleum is in line with the reports of She et al. (2012). This might have been the result of an increase in hydrocarbon utilizing bacteria present in the soil. The low concentrations of crude oil were not enough to cause any significant toxicity effect to the non-hydrocarbon degraders and this led to a slight increase in the total bacterial population.

The inhibitory effects of high levels of crude oil on the microflora as depicted in Figures 1 and 2 conform with results reported in the work of Boethling and Alexander (1979) though not in the soil habitat. This shows that even though it has been demonstrated that microbial communities can affect pollutants (through biodegradation), the presence of pollutants can also affect microbial community size. According to Long et al. (1995) pollutants can alter the community structure through selection of pollutant degraders or through acute toxicity to microorganisms.

From 2.5% crude oil contamination, there occurred a sharp decline in soil bacterial numbers (Figure 1) from the first week up to the fourth week. Ebuehi et al. (2005) during a bioremediation study observed a sharp decline in the population of total heterotrophic bacteria within the first two weeks of their experiment. The oil also reduced the rate of soil respiration (microbial respiration) in a dose-dependent manner even though the effect was more prominent during the first four weeks (Figure 4). This was probably because the induction of hydrocarbon degradation had not yet taken place that time.

After the fourth week of this study, the population rose gradually till the eighth week. The initial reduction in population might be the result of a decrease in the number of non-hydrocarbon degraders which are usually the predominant group in pristine habitats. An

introduction of a hydrocarbon pollutant will severely decrease their population. On the other hand, the crude oil will selectively enrich the soil in favour of hydrocarbon degraders whose catabolic machinery will be activated leading to utilization of the contaminant and a gradual rise in their own population. According to Delille and Delille (2000), hydrocarbon degraders can occur in pristine habitats as a result of the presence of biowaxes derived from vascular plants.

Results on the relative abundance of Gram positive and Gram negative bacteria (Figure 3) in the soil samples showed that Gram positive bacteria predominated over Gram negatives in the unpolluted (control) samples. In the test samples (polluted soils) however, there was a prevalence of Gram negatives over Gram positives. The reason for these differences may be because of the complexity of Gram negative bacterial cell wall which might hinder the penetration of certain substances and their entrance into the cytoplasm. For instance Gram negative bacterial cell walls possess porins which help in the uptake of certain substances by the cell or extrusion of others which may be harmful (Singleton, 1997).

There was a progressive decrease in fungal numbers in all crude oil concentrations as time elapsed (Figure 2). Obayori et al. (2008) however reported an increase in fungal numbers following petroleum pollution during their work on a related subject. Differences in soil physicochemical properties might have contributed to the difference in the two results. The higher susceptibility of fungi than bacteria to crude oil observed in this study might have been caused by the higher adaptability of bacteria than fungi to changes in environmental conditions. Bacterial genomes encode a number of resistance factors which may assist them in circumventing the toxicity effects of pollutants. Moreover, bacteria are the most common group of microorganisms involved in crude oil degradation (Rahman et al., 2003) since they contain various degradative enzymes which catalyze the metabolism of oil hydrocarbons.

Conclusion

This work has been able to highlight the influence of concentration of polluting oil on the population sizes of bacterial and fungal communities in a sandy loam soil habitat. Crude oil can positively or negatively affect microbial population in the soil; the direction of this influence is crude oil–dose dependent as shown in the results of this work. Also reported in this work is the higher vulnerability of fungi than bacteria to crude oil toxicity and the preponderance of Gram negative bacteria over Gram positives in crude oil polluted sandy loam soil. Result such as this, depicting the effects of Bonny light crude oil contamination of sandy loam soil on the microflora has not to our knowledge been reported in previous works. These findings will help in the design of bioremediation strategies for crude oil contaminated sandy loam soils.

REFERENCES

Adoki A, Orugbani T (2007). Influence of nitrogenous fertilizer plants effluents on growth of selected farm crops in soils polluted with crude petroleum hydrocarbons. Afr. J. Agric. Res. 2(11):570.

Atlas RM, Bartha R (1973a). Abundance, distribution and oil degradation potential of microorganisms in Raitan Bay. Environ. Pollut. 4:291-300.

Atlas RM (1977). Simulated petroleum biodegradation. Crit. Rev. Microbiol. 5:371-386.

Boethling RS, Alexander M (1979). Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Appl. Environ. Microbiol. 37:1211-1216.

Chi Yuan F, Krishnamurthy M (1995). Enzymes for enhancing bioremediation of petroleum – contaminated soil: A brief review. Air Waste Manage. Assoc. 45:453-460.

Delille D, Delille B (2000).Field observations on the variability of crude oil impact on indigenous hydrocarbon-degrading bacteria from subantarctic intertidal sediments. J. Mar. Environ. Res. 49(5):403-417.

Ebuehi OA, Abibo IB, Shekwolo PD, Sigismund KI, Adoki A, Okoro IC (2005). Remediation of crude oil contaminated soil by enhanced natural attenuation technique. J. Appl. Sci. Environ. Manage. 9(1):103-106.

Funke BR (1998). Study guide for Microbiology (6th ed). Benjamin/Cummings Co. Inc. California, p. 1.

Long SC, Aelion CM, Dobbins DC, Ptaender FK (1995). A comparison of microbial community characteristics among petroleum-contaminated and uncontaminated sub-surface soil samples. J. Microb. Ecol. 30:297-307.

Millioti VS, Servulo LC, Sobral LG, De Carvalho DD (2009). Bioremediation of crude oil bearing soil: Evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency. Glob. Nest J. 11(2):181-188.

Obayori OS, Ilori MO, Adebusoye SA, Amund OO, Oyetibo GO (2008). Microbial population changes in tropical agricultural soil experimentally contaminated with crude petroleum. Afr. J. Biotechnol. 7:4512-4520.

Odu CTI (1972). Microbiology of soils contaminated with petroleum hydrocarbons, extent of contamination and some soil and microbial properties affected after contamination. J. Inst. Pet. 58:201-208.

Odu CTI (1978). The effects of nutrients application and aeration on oil degradation in soil. Environ. Pollut. 15:235-240.

Okpokwasili GC, Odokuma LO (1990). Effect of salinity on biodegradation of oil spills dispersants. Waste Manag. 10:141-146.

Onuoha CI, Arinze AE, Ataga AE (2003). Evaluation of growth of some fungi in crude oil polluted environment. Glob. J. Agric. Sci. 2:1596-1603.

Pezeshki SR, Hester MW, Lin Q, Nyman JA (2000). The effects of oil spill and clean-up on dominant US Gulf Coast marsh macrophytes: A review. Environ. Pollut. 108:129-139.

Racine CH (1993). Long-term recovery of vegetation on two experimental crude oil spills in interior Alaska black spruce taiga. Can. J. Bot. 72:1171-1177.

Rahman KS, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat IM (2003). Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Biores. Technol. 90:159-168.

She W, Yao J, Wanq F, Cai MM, Wanq JW, Sonq CS (2012). A combination method to study the effects of petroleum on soil microbial activity. Bull. Environ. Contam. Toxicol. 90(1):34-38.

Singleton P (1997). Bacteria in Biology, Biotechnology and Medicine, John Wiley and Sons, New York (4th ed.). pp. 19-20.

Sparrow SD, Sparrow EB (1988). Microbial biomass and activity in a subarctic soil ten years after crude oil spills. J. Environ. Qual. 17:304-309.

Stotzky G (1965). Microbial respiration. In: Black CA(ed.). Methods of soil analysis (Part 2). American Society of Agronomy Inc.,Wisconsin.

Terrence HB, Etienne Y, Christine M, David J, Lyle GW, Charles WG (2011). Identification of nitrogen-incorporating bacteria in petroleum contaminated arctic soils by using ^{15}N DNA-based stable isotope probing and pyrosequencing. Appl. Environ. Microbiol. 77(12):4163-4171.

Wistreich GA (1997). Microbiology Laboratory: Fundamentals and Applications. Prentice-Hall Inc. New Jersey, USA. pp. 144-186, 194-259.

Wyszkowska J, Kucharski J, Jastrzebska E, Hiasko A (2001). The biological properties of the soil as influenced by chromium contamination. Polish J. Environ. Stud. 10:37-42.