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Variation in the Carbon (C), Phosphorus (P) and Nitrogen (N) Utilization during the Biodegradation of Crude Oil in Soil

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Abstract

This study was aimed at determining the effect of varying concentration of crude oil pollution on the macro-nutrients in the soil. Various macro-nutrients (such as soil ammonium concentration, soil nitrate concentration, and available phosphorus), oxidizable organic carbon, and total petroleum hydrocarbon were determined. The result revealed that as the time increases, the oxidizable carbon, which is also a function of the organic matter decreases, which is as a result of the conversion of carbon to carbon (IV) oxide during cellular metabolism. This decrease showed that there was an increase in the activity of that leads to the breakdown of the carbon components in the soil. The soil phosphate concentration determination did not show any pattern in their increase or decrease, which shows that increase in crude oil concentration, did not significantly affect the phosphate concentration in the soil. The soil ammonium concentration increased from 24th hour to 168th hour but decreased before the end of the experiment. This increase could be attributed to ability of the Azotobacter *vinelandii* to fixed nitrogen as an innate responsibility, while *Pseudomonas* sp. which is known to contain nitrogen fixing genes. The result also showed that there is a constant increase in soil nitrate concentration which is affected as the concentration of the pollutant (crude oil) increases. This study has shown that the consortium of these organisms can be used as a bio-fertilizer as well as in bioremediation.

Keywords: Bioremediation; Ammonium; Nitrate; Phosphorus; *Pseudomonas* and *azotobacter*

Introduction

Crude oil is a complex mixture of various hydrocarbons, which are made up various aliphatic and aromatic hydrocarbons [1]. They also contain poly aromatic hydrocarbons (PAHs) which are recalcitrant. The dependency of crude oil as a major source of energy in Nigeria, has led to the pollution of different environment (land/soil, water and air). The causes of these pollutions include; exploration, exploitation, storage, transportation, vandalization, bunkering and gas flaring [2]. These have led to the introduction of various pollutants into the environments that have been implicated in the depiction of microbial flora in the soil, death of aquatic organisms, the depletion of the ozone layer and the formation of acid rain. Most of these pollutants such as the PAHs have been implicated in cancer, mutation etc [3]. In the soil and land environment, crude oil pollution has been known to affect agricultural yield as it hinders plant growth. Due to these effects of crude oil pollution, it then becomes necessary that oil spills are cleaned up as quickly as possible. One of the methods used in cleanup of the environment is the use of microorganism or products of microorganism known as bioremediation. The essence of bioremediation is not just to remove the pollutants, it also to restore the environment to its habitable form. Therefore physical methods of remediation are not employed. *Pseudomonas* species have been employed in the remediation of crude oil environment [4] due to its ability to biodegrade crude oil using it as a carbon source for generation of biomass and energy. But for the organisms to carry out their cellular activity, the supply of other macronutrient such as nitrogen and phosphorus are very necessary. Nitrogen been are major component of amino acid, purines and pyrimidines is required for the formation of proteins. RNAs and DNAs [5]. These control the functions of the organism to a large extent. Phosphorus have been involved in the formation of phospholipid which are involved in cell wall formation and also in the production of energy carriers, such as ATP, GTP, UTP, TTP, CTP etc. *Pseudomonas* are known to solubilize phosphorus from the soil [6], while Azotobacter *vinelandii* fix atmospheric nitrogen into the soil [7], both organisms thereby act as a biofertilizer for the supply of these macronutrient. Therefore this study is aimed at determining how different concentration of crude oil in soil can affect carbon, nitrogen and phosphorus content in the soil.

Materials and Method

Crude oil

The Crude oil used was gotten from the Directorate of Petroleum Resources Port-Harcourt, Rivers State Nigeria,

Soil

The soil samples that were used in this study were obtained from the Agric Farm, Department of Agriculture, University of Nigeria, and Near Green House.

Microorganism

Two microorganisms were used in the course of the research. The *Pseudomonas* species was gotten from the culture collection Centre Department of Microbiology, University of Nigeria, Nsukka while the Azotobacter *vinelandii* was isolated from the soil around the

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postgraduate laboratory, Department of Biochemistry, University of Nigeria, Nsukka using Azotobacter *vinelandii* specific media.

Determination of the remaining Total Petroleum Hydrocarbon (TPH) the modified methods of Ubani et al.

One gram (1 g) of soil was put in a test-tube and ten milliliter (10 ml) of Chloroform/Ethanol mixture (1:1) was added. The mixture was agitated for 5 minutes and then allowed to stand for 10 minutes. The sample was then filtered and the absorbance of the filtrate was taken at 520 nm using chloroform/Ethanol mixture (1:1) as a blank. The quantity of crude oil was estimated using a crude oil standard curve.

Determination of soil percentage oxidizible organic carbon, Total Organic Carbon and Organic Matter

Two grams (2 g) of dried soil was transfer to a 500 mL Erlenmeyer flask, and 10 mL of 0.167 M $K_2Cr_2O_7$ was added by means of a pipette. 20 ml of concentrated H_2SO_4 was and swirl gently to mix, (Excessive swirling was avoided to prevent the organic particles from adhering to the sides of the flask out of the solution). The mixture was allowed to stand on an insulation pad for about 30 minutes. Then 200 ml of distilled water was used to dilute the suspension so as to provide a clearer solution for viewing the endpoint. Then 10 ml of 85% H_3PO_4 was added and 0.2 g of NaF was also added. The H_3PO_4 and NaF are added to complex Fe^{3+} which would interfere with the titration endpoint. 10 drops of ferroin indicator was added and then titrated with 0.5 M Fe^{2+}. The color of the solution at the beginning is yellow-orange to dark green, depending on the amount of unreacted $Cr_2O_7^{2-}$ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint.

The organic carbon and organic matter percentages where calculated thus:

a. Percentage easily oxidizable organic C

$$\%C=\frac{(B\text{-}S)\times M \text{ of } Fe^{2+} \times 12 \times 100}{\text{grams of soil} \times 4000}$$

B = ml of Fe^{2+} solution used to titrate blank

S = ml of Fe^{2+} solution used to titrate sample

12/4000 = milliequivalent weight of carbon in grams

To convert easily oxidizable organic C to total C, divide by 0.77 (or multiply by 1.30) or other experimentally determined correction factor.

b. Percentage organic matter (OM)

$$\%\ OM=\frac{\%C}{0.58}=\%C \times 1.72$$

Determination of phosphorus concentration (Ascorbic Acid Method Procedure)

Two grams (2 g) of soil was weighed in a boiling tube, 20 ml of the extracting solution was added and the soil mixture was agitated for 5-10 mins. It was allowed to stand for 30 mins with occasional agitation every 8 mins. At the end of the 30 mins, it was filtered and the filtrate was collected. 2ml of the filtrated was then. Add to 8 mL of working solution in a test – tube. It was thorough agitation and mixing occurs. The mixture was allowed to stand for 10 minutes for color development before taken the absorbance at 882 nm. Read percentage of transmittance or optical density on a colorimeter or spectrophotometer set at 882 nm. Color is stable for about 2 hours.

A standard curve was prepared by pipetting a 5 ml aliquot of each working standard, developing color and reading absorbance in the same manner as with the soil extracts. The absorbance was plot against concentration of working standards. The concentration in soil extracts was determined from absorbance and the standard curve.

Determination of soil ammonium

One milliliter 1 mL of solution soil extract was put into a test-tube; 5.5 ml of buffer solution was added and agitated for 5 minutes. 4 ml of salicylate/nitroprusside solution was added and also 2 ml of hypochlorite solution was added and mixed properly. The mixture was allowed to stand for 45 mins at 37°C. The absorbance was taken at 650 nm with 2 hours.

Determination of soil nitrate

Twenty grams (20 g) of soil was weighed into a 100 ml beaker and 50 ml of extracting solution was added. It was agitated for 5 minutes and the potential was read in millivolts (mV) using a millivolt meter, while the mixture is being stirred. The concentration of NO_3 - N, was determined using the standard curve.

Record the millivolt reading (if using a calibration curve technique) or read the NO_3–N concentration directly from a pH/ion meter.

Results and Discussion

The use of micro-organisms to decontaminate the environment (Bioremediation), is being increasingly seen as an effective, environment- friendly treatment for crude oil contaminated sites. Large quantities of organic and inorganic compounds are released into the environments every year as results of anthropogenic activities thereby causing serious environmental problems [8]. The result of this study reveals Azotobacter *vinelandii* possesses the ability to breakdown crude oil in the soil (Figure 1). Azotobacter *vinelandii* is an autotroph which has the natural ability to fix atmospheric nitrogen into the soil and improve soil fertility [9]. This it does with the aid of nitrogenase complex (EC1.18.6.1) [10]. Some Azotobacter sp. such Azotobacter *chroococcum* has been reported to breakdown crude oil, emulsifies waste motor oil and other fractions petroleum indicating its potentiality in utilization of various hydrocarbons [11]. Azotobacter sp are not known to possess the ability to breakdown crude oil but they have the ability to pick up plasmids from the environment [12], which confers on them the properties which they naturally do not possess. Most adapted strains of Azotobacter sp possess the ability to breakdown crude oil. Figure 1 also showed that the rate of breakdown

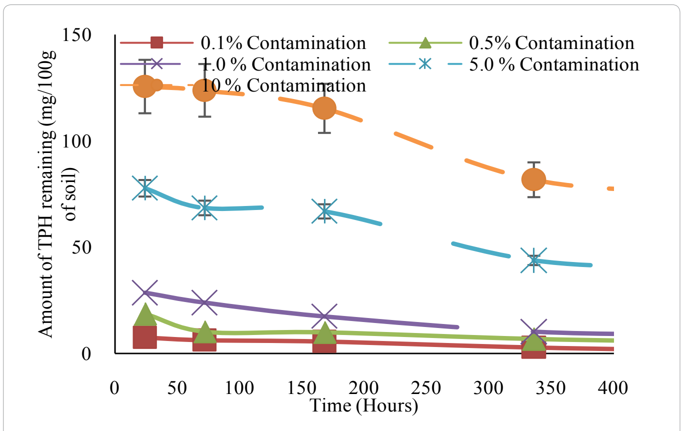

Figure 1: The concentration of TPH in the soil containing *Azotobactervinelandii*.

of crude oil was low in the first 196 hrs. of the experiment; this is due to the time taken for the organism to produce the enzymes necessary for the breakdown of crude oil.

The TPH concentration in the soil seeded with *Azotobacter vinelandii*

Figure 1 shows the amount of total petroleum hydrocarbon (TPH) that is remaining in the soil. The figure shows that there is a gradual decrease in the TPH concentration with time. This decrease was found in all the groups from 0.1% contamination to 10.0% contamination.

The concentration of TPH in the soil containing *Pseudomonas* sp in Figure 2 showed that there is also a decrease in the amount of TPH in the soil with time. This is because of the ability of *Pseudomonas* sp to utilize crude oil as a carbon source [13,14].

During this process, biosurfactants are produced which helps to reduce the surface tension of crude oil thereby making the oil to be soluble in aqueous solution thereby making the crude oil available for microbial attack. *Pseudomonas* also produces lipase which helps in the degradation of lipids [15].

It have been reported that *Pseudomonas* sp possesses genes that code for enzymes such as catechol dioxygenase, alkane 1-monooxygenase and alkane sulfonate monooxygenase [16] that help in the breaking down of hydrocarbon chains.

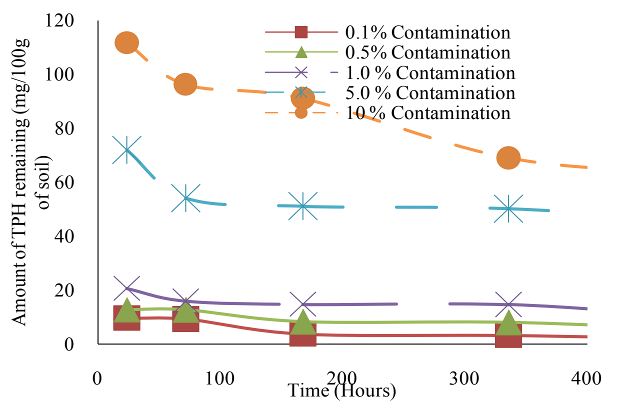

Figure 2: The concentration of TPH in the soil containing *Pseudomonas sp.*

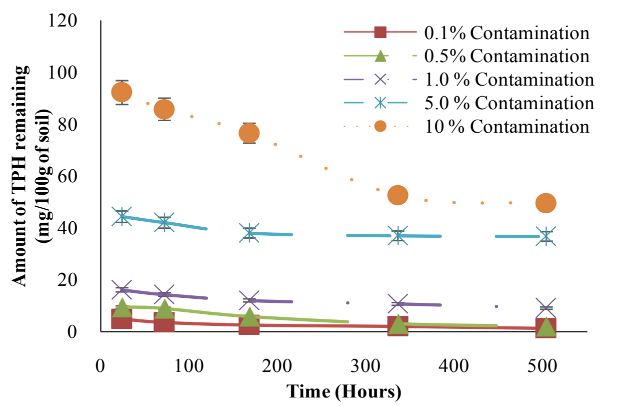

Figure 3: The concentration of TPH in the soil containing a consortium of *Azotobactervinelandii* and *pseudomonas sp.*

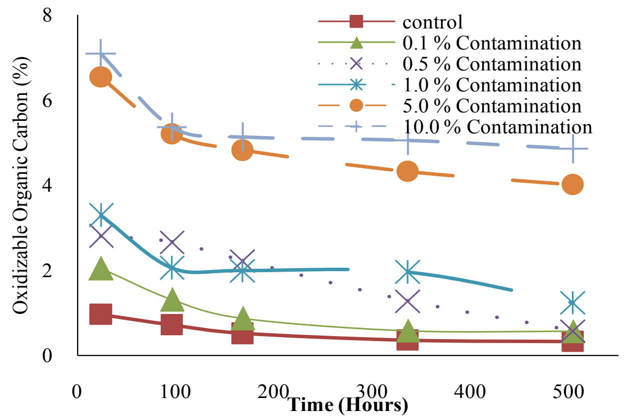

Figure 4: The Percentage Oxidizable Organic Carbon in soil containing *Azotobactervinelandii.*

The result also showed that *Pseudomonas* possess the ability to breakdown high concentration of crude oil thereby withstanding to some extent the toxicity of crude oil.

The concentration of TPH in the soil containing *Pseudomonas* in Figure 3 showed that there is also a decrease in the amount of TPH in the soil with time. The result shows that the consortium broke down more of the pollutants (crude oil) when compared with the individual organisms. This is as a result of the synergism that exist between the organisms [17].

The percentage oxidizable organic carbon in soil containing *Azotobacter vinelandii.*

The percentage of oxidizable organic carbon was observed to increase as the concentration of crude oil in the soil increases due to the fact that crude oil contains oxidizable carbons. As a result, the percentage of oxidizable carbon can be used to a parameter to determine the degradation ability of the microorganism [18,19].

Figures 4-6 show the gradual decrease in the percentage of oxidizable carbon as the time increases. It was also observed that the rate of decrease was high within the first 196 hrs of the experiment which is also corresponding to the period of rapid growth of the organisms. It could also be observed that at high concentration of the crude oil (10%, 5% and 1% contamination) there was a decrease in the rate of utilization of the oxidizable carbon (Figure 4). This might be as a result of the presence of some recalcitrant polyaromatic hydrocarbon PAH in the crude oil which might to be easily degraded be the organisms such as Azotobacter *vinelandii* or that the toxicity of crude oil increases with increase in percentage of crude oil contamination which might inhibit the metabolic activities in the organisms. Azotobacter *sp* are used as biofertilizers due to their ability to utilize oxidizable carbon for energy and fix nitrogen in the soil [20]. Probably, this may be the reason why Azotobacter sp is readily found in the soil environment.

The percentage oxidizable organic carbon in soil containing *Pseudomonas sp.*

The Figure 5 reveals a decrease in the percentage of oxidizable organic carbon in the soil containing *Pseudomonas* as was observed in Figure 4.

It was also observed that there was a continuous reduction in percentage of oxidizable carbon. Some species of *Pseudomonas* have

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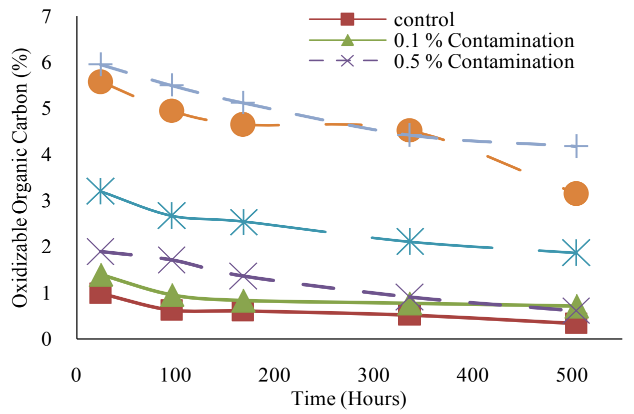

Figure 5: The Percentage Oxidizable Organic Carbon in soil containing *Pseudomonas sp.*

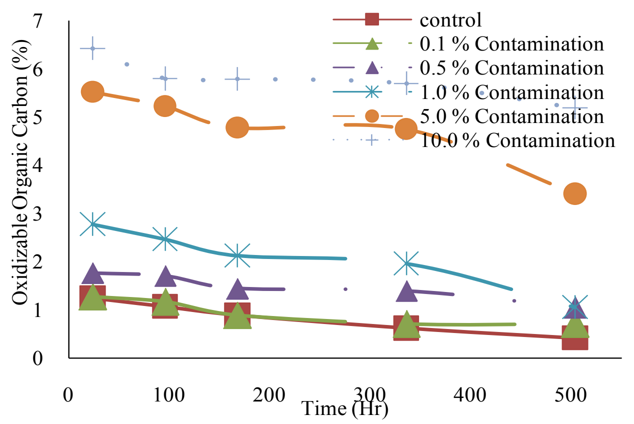

Figure 6: The Percentage Oxidizable Organic Carbon in soil containing a consortium of *Azotobactervinelandii*and *Pseudomonas sp.*

been found to remove organic carbon during denitrification reactions using them as electron donors during the process [21]. *Pseudomonas* sp have been known to breakdown long chain hydrocarbon utilizing them for growth and energy [22], therefore the continuous reduction in the percentage of oxidizable organic carbon could also be attributed to this ability the was naturally conferred on *Pseudomonas* sp.

The percentage oxidizable organic carbon in soil containing a consortium of *Azotobacter vinelandii* and *Pseudomonas sp.*

Figure 6 show the levels of oxidizable organic carbon in the soil containing a consortium of Azotobacter *vinelandii* and *Pseudomonas* sp. and the result reveals that there was a reduction in the organic carbon as the level of contamination increase although the result shows that at higher concentration of crude oil, that the change in the level of oxidizable carbon did not change significantly. Rathore [23] has shown that there is an existing synergism existing between *Azotobacter sp* and *Pseudomonas* sp in promoting the growth of plants. Therefore, one would have expected synergism between the organisms in reducing the oxidizable organic carbon but rate of reduction was not quite different when compared with the individual organisms. This probably show that to some extent that there are some level of inhibition existing between the organisms even though it did not affect the growth of the organism due to the fact that both organisms could be depending on different substrates for energy and growth in which one organism might be depending on the product of the second organism.

The soil phosphate in soil containing *Azotobacter vinelandii*

The result in Figure 7 which shows the available soil phosphate concentration in the soil containing *Azotobacter vinelandii* revealed increasing concentration of phosphate in all the groups after 168 hours but decreased gradually in all the levels of contamination before 336 hours. The increase in phosphorus concentration also increases with in biomass (growth pattern) of the organism. Therefore as the biomass (*Azotobacter vinelandii*) increases, soil phosphate concentration increases because the organism possesses genes for phosphate solublization, even though they are expressed at a reduced rate. The initial rise in the soil available phosphorus was due to the ability of organism to solubilize phosphate in the soil. But as the concentration of phosphorus in the environment increases, there is a feedback inhibition of phosphatase activity by phosphate [24] which inactivates phosphorus solubilization.

The soil phosphate in soil containing *Pseudomonas sp*

The result in Figure 8 which shows the available soil phosphate concentration in the soil containing *Pseudomonas* sp. revealed higher

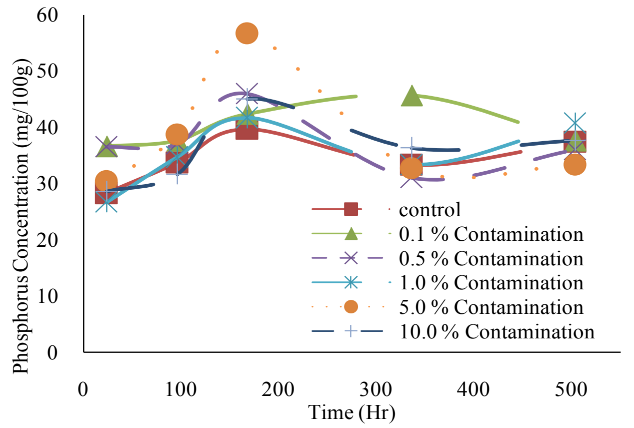

Figure 7: The concentration of Phosphate in soil containing *Azotobactervinelandii.*

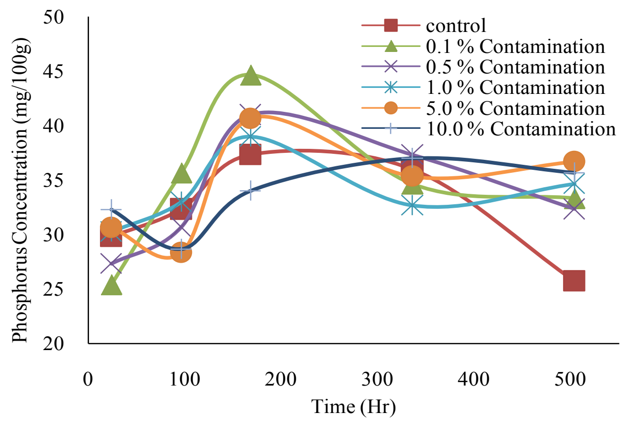

Figure 8: The concentration of Phosphate in soil containing *Pseudomonas sp.*

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concentration of phosphate in all the groups after 168 hours but decreased gradually in all the levels of contamination. *Pseudomonas* sp are known to solubilize phosphate [6,25,26].

At the 168 hour, the concentration of available phosphate was lowest in soil sample with 10% contamination. This could be attributed to the toxicity of the pollutant. Also the control showed low available phosphate which could be attributed to the concentration of carbon source. According to Isolation and characterization of phosphate solubilizing bacteria (Klebsiella oxytoca) with enhanced tolerant to environmental stress [27].

The soil phosphate in soil containing *Azotobacter vinelandii* and *Pseudomonas sp*

The results (Figure 9) also show that there was an increase in the control group, 0.1% up to 168 hr and then the decrease sets in gradually. The variation at the 24th hour might be as a result of the variations in the localization of phosphate in the soil. But on the general note, it was observe that available concentration of phosphorus increase till the 168hr as was observed in the previous charts before a gradual decrease which could be attributed to microbial utilization of the free phosphorus.

The ammonium concentration in soil containing *Azotobacter vinelandii*

The concentration of the soil ammonium was found to increase in all the group up to about 96hours and then a gradual decrease was observed although the decrease did not follow a sequential other as seen in Figure 10.

The ammonium concentration in soil containing *Pseudomonas sp.*

The result in Figure 11 shows a sharp increase in the ammonium concentration in the soil from 24hours to 96 hours and after 168 hours, a gradual decrease was observed.

The ammonium concentration in soil containing a consortium of *Azotobacter vinelandii* and *Pseudomonas sp.*

Figure 12 also shows an increase in ammonium concentration in the soil from 24hour to 168 hour in all the groups but a sharp decrease was observed between 336 hour to 504 hours in all the levels of contamination.

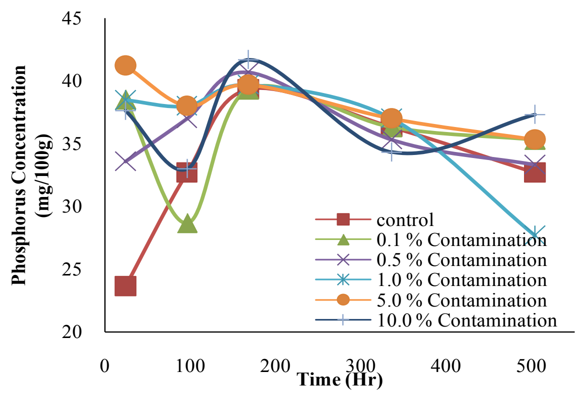

Figure 9: The concentration of Phosphate in soil containing a consortium of *Azotobactervinelandii*and *Pseudomonas sp.*

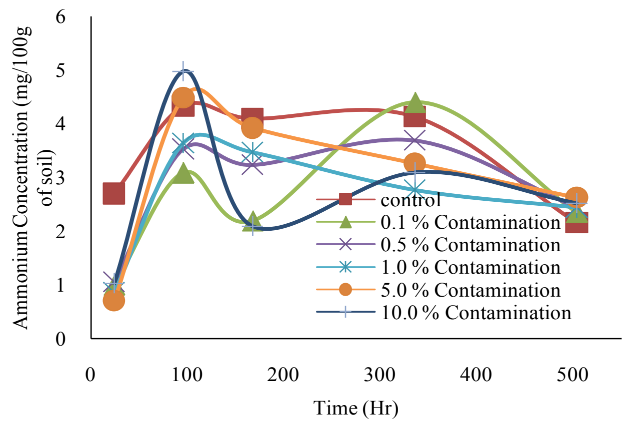

Figure 10: The concentration of Ammonium in soil containing a consortium of *Azotobactervinelandii.*

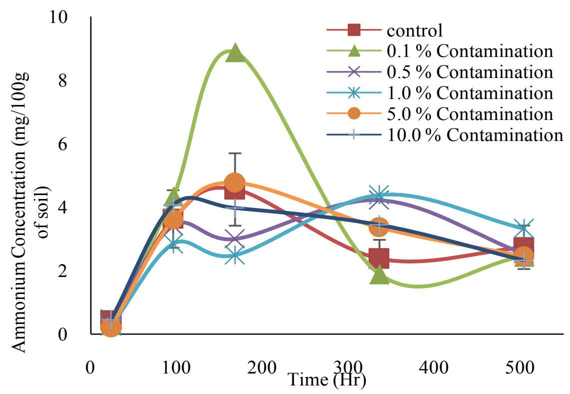

Figure 11: The concentration of Ammonium in soil containing *Pseudomonas sp.*

The nitrate concentration in soil containing *Azotobacter vinelandii*

The result of Figure 13 show that there was an increase in the nitrate concentration as the time increases but decreased with an increase in the percentage contamination.

The nitrate concentration in soil containing a consortium of *Pseudomonas sp.*

Figure 14 shows that there was a decrease in the nitrate concentration as the levels of contamination increase that is from 0.1% crude oil contamination to 10.0% crude oil contamination. It was also observed that the nitrate concentration increased with time. Although there is an unusual decrease at 5% crude oil contamination.

The nitrate concentration in soil containing a consortium of *Azotobacter vinelandii* and *Pseudomonas sp.*

Figure 15 above showed that there was a decrease in the soil nitrate concentration as the percentage of crude oil contamination increases. On the other hand, it was observed that was an increase in the nitrate concentration of the soil containing the consortium as the time increases. Although at the 336th hour in the consortium, there

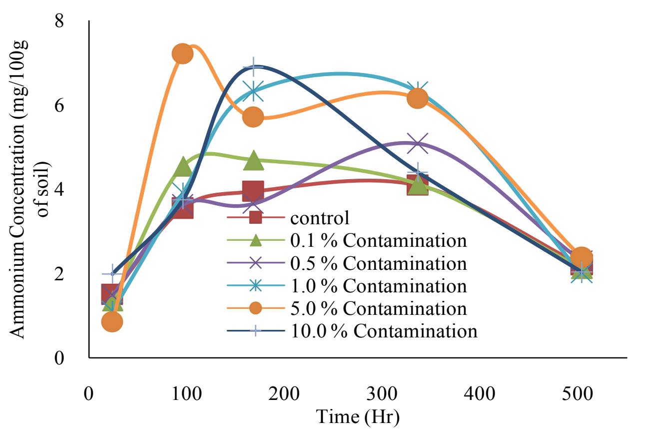

Figure 12: The concentration of Ammonium in soil containing a consortium of *Azotobactervinelandii*and *Pseudomonas sp.*

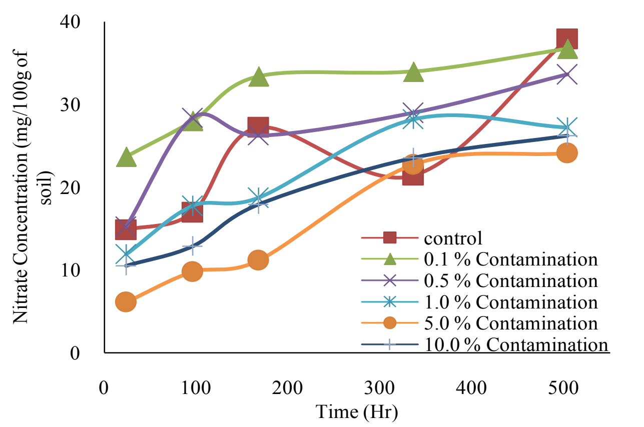

Figure 13: The concentration of nitrate in soil containing *Azotobactervinelandii.*

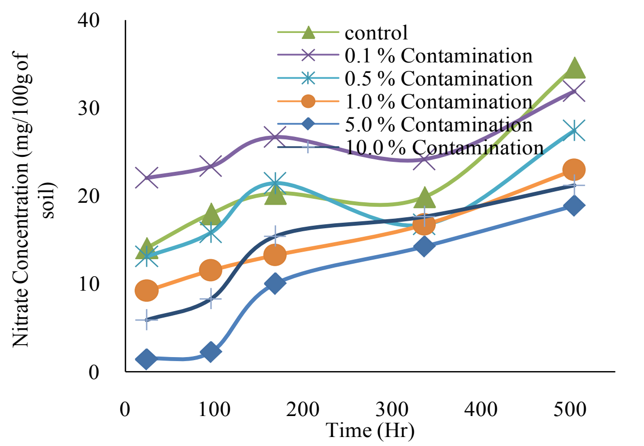

Figure 14: The concentration of nitrate in soil containing *Pseudomonas sp.*

was an observed decrease in the nitrate concentration which might be as attributed to increase in the rate at which nitrate is utilized by the organism at the period which consider with the period of maximum growth. But as the amount of organisms begin to reduce, the rate of

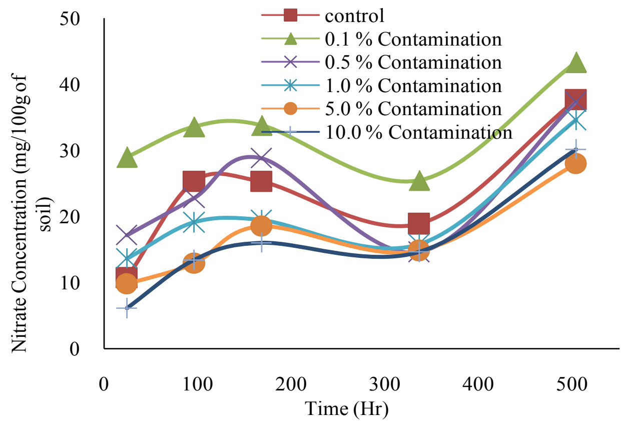

Figure 15: The concentration of nitrate in soil containing a consortium of *Azotobactervinelandii*and *Pseudomonas sp.*

utilization begins to reduce giving room to more nitrate in the soil.

Conclusion

Soil organic matter is made up of plant nutrients and carbon sources. Organic matter in soil are from natural materials such as plant materials, animal litters and microbial biomass. Soil organic matter influences the bioavailability of nutrients as well as soil enzymes produced by bacteria. Bioavailability is one of the most limiting factors in bioremediation of persistent organic pollutants in soil. Compared to the other soil characteristics, soil organic matter is the major factor which affects the distribution and bioavailability of petroleum hydrocarbons. The result revealed that as the percentage of contamination increases, there is also an increase in the above mentioned parameters. According to Liu et al., Soil organic matter increased significantly after an oil contamination. It was also observed that as the time (days) increases, the oxidizable carbon, total organic carbon and the organic matter decreases. This is as a result of the conversion of carbon to carbon (IV) oxide during cellular metabolism which is released into the atmosphere. It was observed in most cases, that the *A. vinelandii* showed lower concentration of the organic carbon when compared with *Pseudomonas* sp and the consortium. The decrease might be as a result of increase in the activity of that leads to the breakdown of the carbon components of the soil, even though there was low microbial growth. This might also be due to the large size of organism which might require more carbon for macromolecule synthesis.

The soil phosphate concentration was determined and the result did not show any pattern in their increase or decrease. This shows that increase in crude oil concentration did not significantly affect the phosphate concentration in the soil. *Pseudomonas* sp is known to solubilize phosphate in the soil and make them available. Azotobacter according to Rediers et al., should be classified under the genus as *Pseudomonas* since both of them have a lot of gene sequence in common. It is therefore possible that A. vinelandii also solubilizes phosphate in the soil and this should account for the slight increase in the level of phosphate found in the consortium.

Most *Pseudomonas* species are known for their ability to reduce of nitrates back into the largely inert nitrogen gas (N2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and Clostridium in anaerobic conditions. They use the nitrate as an electron acceptor in the place of oxygen during respiration. Some microorganisms possess some ability to undergo transformation especially in extreme conditions such extreme pH, temperature, and deficiency of nutrients. In aerobic conditions, some *Pseudomonas* species can help fix nitrogen by converting atmospheric nitrogen to ammonium and then to nitrate. The nif-Hphylogenies (which is responsible for fixing nitrogen is found in *Pseudomonas*) contains at least 42 genes to encode the denitrification apparatus' core structures. The genome also contains genes involved in nitrogen fixation, denitrification, chemotaxis and other functions that presumably give the *Pseudomonas* an advantage. The result of this research shows that the soil ammonium concentration increased from day 1 to day 7 but decreased before day 21. This increase could be attributed to ability of the Azotobacter *vinelandii* to fixed nitrogen as an innate responsibility, while *Pseudomonas* sp. which is known to contain nitrogen fixing activity. The increase observed tallies with the growth pattern of the organisms which shows that as the organism decrease in number, there is a decrease in the amount of ammonium in the soil. Although it should be noted that the organisms still use some quantity of nitrogen for cellular activity.

The result also showed that there is a constant increase in soil nitrate concentration from day 1 to 7 but this decrease with increasing concentration of the pollutant (crude oil). This shows that increase in crude oil also reduces nitrate concentration in the soil. At high concentration of crude oil, cellular activities are distorted due to toxicity introduced by the crude oil.

The result of this study showed a constant decrease in the percentage of organic carbon (OC) and the total petroleum hydrocarbon (TPH). This is evidence that organisms *Pseudomonas* and *Azotobacter vinelandii* have the ability to utilize the TPH as an alternative source of energy therefore able to remove this contamination and restoring the environment. It was observed that there was an increase in macro-nutrients (nitrogen and phosphorus) which are needed to improve the soil fertility. Therefore a consortium of the *Pseudomonas* spp and *Azotobacter vinelandii* can be used not only in the remediation but also as a bio-fertilizer.

References

1. Ghazali FM, Rahman RN, Salleh AB, Basri M (2004) Biodegradation of hydrocarbon in soil by microbial consortium. International Biodeterioration and Biodegradation 54: 61-67.
2. Ite AE, Ibok UJ, Ite MU and Petters SW (2013) Petroleum Exploration and Production: Past and Present Environmental Issues in the Nigeria's Niger Delta. American Journal of Environmental Protection 1: 78-90.
3. Le T, Qureshi A, Heisler J, Bryant L, Shah J, et al. (2014) Polycyclic aromatic hydrocarbons and small related molecules: Effects of Schizosacharomyces pombe morphlogy measured by imaging flow cytometry. Journal of Yeast and Fungal Research 5: 84-91.
4. Al-Wasify RS, Hamed SR (2014) Bacterial Biodegradation of Crude Oil Using Local Isolates. International Journal of Bacteriology 2014: 1-8.
5. Katoch R (2011) Qualitative and Quantitative Estimations of Amino Acids and Proteins. Analytical Techniques in Biochemistry and Molecular Biology 93-147.
6. Delgado M, Mendez J, Rodríguez-Herrera R, Aguilar CN, Cruz-Hernández M, et al. (2014) Characterization of phosphate-solubilizing bacteria isolated from the arid soils of a semi-desert region of north-east Mexico. Biological Agriculture and Horticulture: An International Journal for Sustainable Production Systems 30: 211-217.
7. Swain H, Abhijita S (2013) Nitrogen Fixation and Its Improvement through Genetic Engineering. Journal of Global Biosciences 2: 98-112.
8. Battikhi MN (2014) Editorial: Bioremediation of Petroleum Sludge. J Microbiol Exp 1: 00011.
9. Onwurah INE, Ogugua VN, Onyike NB, Ochonogor AE, Otitoju OF (2007) Crude Oil Spills in the Environment, Effects and Some Innovative Clean-up Biotechnologies. International Journal of Environmental Research 1: 307-320.
10. Seyhan E, Kirwan DJ (1979) Nitrogenase activity of immobilized Azotobacter vinelandii. Biotechnol. Bioeng 21: 271-281.
11. Thavasi R, Jayalakshmi S, Balasubramanian T, Banat IM (2006) Biodegradation of Crude Oil by Nitrogen Fixing Marine Bacteria Azotobacter chroococcum. Research Journal of Microbiology 1: 401-408.
12. Guo C, Sun L, Kong D, Sun M, Zhao K (2014) *Klebsiella variicola*, a nitrogen fixing activity endophytic bacterium isolated from the gut of *Odontotermes formosanus*. African Journal of Microbiology Research 8: 1322-1330.
13. Chikere CB, Ekwuabu CB (2014) Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoni land, Nigeria. African Journal of Environmental Science and Technology 8: 401-406.
14. Almansoory AF, Idris M, Abdullah SRS, Anuar N (2014) Screening For Potential Biosurfactant Producing Bacteria From Hydrocarbon Degrading Isolates. Advances in Environmental Biology 8: 639-647.
15. Azhdarpoor A, Mortazavi B, Moussavi G (2014) Oily wastewaters treatment using Pseudomonas sp. isolated from the compost fertilizer. Journal of Environmental Health Science & Engineering 12: 77.
16. Patel PA, Kothari VV, Kothari CR, Faldu PR, Domadia KK, et al. (2014) Draft genome sequence of petroleum hydrocarbon-degrading Pseudomonas aeruginosa strain PK6, isolated from the Saurashtra region of Gujarat, India. Genome Announc 2: e00002-14.
17. Anitha G, Kumudini BS (2014) Isolation and characterization of fluorescent pseudomonads and their effect on plant growth promotion. Journal of Environmental Biology 35: 627-634.
18. Mrayyan B, Battikhi MN (2005) Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge. J Hazard Mater 120: 127-134.
19. Okoro SE, Adoki A (2014) Bioremediation of crude oil impacted soil utilizing surfactant, nutrient and enzyme amendments. J Bio Env Sci 4: 41-50.
20. El-Lattief EA (2013) Impact of integrated use of bio and mineral nitrogen fertilizer on productivity and profitability of wheat (*Triticum aestivum* L.) under Upper Egypt conditions. International Journal of Agronomy and Agricultural Research 3: 67-73.
21. Guo H, Chen C, Lee DJ, Wang A, Ren N (2013) Sulfur-nitrogen-carbon removal of Pseudomonas sp. C27 under sulfide stress. Enzyme Microb Technol 53: 6-12.
22. Alsulami AA, Altaee AMR, Al-Kanany FNA (2014) Improving oil biodegradability of aliphatic crude oil fraction by bacteria from oil polluted water. African Journal of Biotechnology 13: 1243-1249.
23. Rathore P (2014) A Review on Approaches to Develop Plant Growth Promoting Rhizobacteria. International Journal of Recent Scientific Research 5: 403-407.
24. Traoré L, Nakatsu CH, DeLeon A, Stott DE (2013) Characterization of six phosphate-dissolving bacteria isolated from rhizospheric soils in Mali. African Journal of Microbiology Research 7: 3641-3650.
25. Rajasankar R, Manju-Gayathry G, Sathiavelu A, Ramalingam C, Saravanan VS (2013) Pesticide tolerant and phosphorus solubilizing Pseudomonas sp. strain SGRAJ09 isolated from pesticides treated Achillea clavennae rhizosphere soil. Ecotoxicology 22: 707-717.
26. Goteti PK, Desai S, Emmanuel LDA, Taduri M, Sultana U (2014) Phosphate Solubilization Potential of Fluorescent Pseudomonas spp. Isolated from Diverse Agro-Ecosystems of India. International Journal of Soil Science 9: 101-110.
27. Walpola BC, Arunakumara KKIU, Yoon M (2014) Isolation and characterization of phosphate solubilizing bacteria (Klebsiella oxytoca) with enhanced tolerant to environmental stress. African Journal of Microbiology Research 8: 2970-2978.