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Research Article

Application of Agro-Waste in Bioremediation of Crude Oil Polluted Soil from Agip Oil Spill Sites at Abecheke Ohaji-Egbema Local Government Area of IMO State

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Abstract

The potential effects of using organic agro-waste as bioremediation material for the detoxification of crude oil contaminated soil from Agip crude oil spill sites at Abecheke Ohaji-Egbema local government area of Imo state was carried out. Cassava and Plantain peels were used by either addition of Cow dung, Poultry droppings or Pig slurry as inoculants. The blending ratio for the samples are A (Soil 2kg, Cassava 3kg, Pig slurry 0.5kg), B (Soil 1.5kg, Cassava 1.5kg, Poultry droppings 0.5kg) C (Soil 1.5kg, Cassava 3kg, Cow dung 0.5kg), D (Soil 2kg, Plantain 3kg, Pig slurry 0.5kg) and sample E (Untreated contaminated sample) which served as control. The samples were isolated into four different zip lock nylon and left for 90 days after which the samples were analyzed for some selected physico-chemical properties. The total hydrocarbon content of the samples reduced from 3657mg/kg in the untreated soil sample to 1282mg/kg in the decreasing order of (E>D>C>B>A) due to the metabolic activities of living organisms involved in the biodegradation process. The mineralization activities of the biodegradation organism improved the pH, nitrogen, moisture and organic carbon content of the treated samples. Electrical conductivity, phosphorus, calcium, magnesium and potassium were reduced due to their utilization by living organisms. The percentage of sand, silt and clay remained the same across all the samples except for percentage of sand in samples B and C that was slightly reduced while the percentage of clay and silt in sample B witnessed slight increment, the texture remained constant across board. Higher level of crude oil biodegradation occurred in cassava peels and pig slurry blend, therefore the use cassava peels mixed with other agro-waste should be encouraged as bioremediation agent of crude oil polluted sites.

Keywords: Crude Oil; Soil Contamination; Hydrocarbons; Bioremediation and Agro-waste

Introduction

Nigeria is one of the largest producers of crude in Africa, the eleventh largest producer of petroleum, the eight largest exporter of crude oil and has the tenth largest proven reserve of crude oil in the world (Akuru and Okoro, 2011). Due to high level of oil exploration activities going on in Nigeria, most oil producing communities in Nigeria are battling with various negative environmental impacts associated with leakages and accidental spills that occur regularly during the exploration, refining, transportation and storage of crude oil and its products (Oyodele *et al*., 2016). Chemically, crude oil is made up of carbon and hydrogen compounds; but also contains nitrogen, oxygen, sulphur, and various metals (Yu *et al*., 2020). It consists of four main components: saturated hydrocarbon, aromatic hydrocarbon, asphaltene, and colloids that pose a significant risk for the environment and human health having mutagenic and carcinogenic effects (Romanus *et al*., 2015). Crude oil spill has caused various degrees of environmental degradation by not only destroying the traditional livelihood of most oil producing region in Nigeria but have also caused environmental pollution that affects weather conditions, soil fertility, waterways, aquatic habitats and wildlife (Onwurah *et al*., 2007). The presence of crude oil in the soil causes soil coagulation thereby decreasing soil porosity and air content, retarding germination rate and decreasing soil nutrient composition causing low crop yield (Udo and Fayemi, 1975). Efforts are ongoing to use various techniques to decontaminate crude oil polluted environments with various remediation methods. Remediation is the process of returning soil, water or air functionality that existed prior to the contamination. It is the removal of hazardous contaminants from soil, groundwater, sediment, surface water, which provides an opportunity to reduce pollution and possible pollution related matters (WHO, 2018). To remedy crude oil polluted soil, variety of techniques exist for soil remediation which includes but not only physical and chemical remediation technique. However, these technologies are cost expensive and can lead to incomplete decomposition of contaminants (Ding *et al*., 2020, Yu *et al*., 2020). Bioremediation is the latest scientific strategy that has been discovered to be cost effective, possess great potential in removing crude oil from the soil and degrade petroleum hydrocarbons without leaving behind any toxic products in the soil ecosystem (Oyodele *et al*., 2016). It involves the use of micro-organisms to facilitate the natural biodegradation process of hydrocarbon through provision of nutrient and oxygen required by micro-organism, crude oil compounds are degraded by micro-organisms with the aid of enzymatic reaction turning them to carbon (iv) oxide, biomass and water soluble compounds (Lim *et al*., 2016, Atlas *et al*., 2015). Most of the physical and chemical properties of the soil, such as aeration, pH, water-holding capacity, and ion exchange capacity can be improved through the process (Nkereuwem *et al*., 2010). Bioremediation of crude oil polluted soil takes two major approach which are biostimulation and bioaugmentation. The former involves the modification of the environment to stimulate existing bacteria capable of bioremediation (Adam, *et al*., 2015). The later involves enhancing microbial performance through addition of genetically engineered/ modified bacteria with specific metabolic activities, which have ability to increase biodegradation (Varjani, 2017).Other environmental growth factors such as pH and temperature are also needed to be controlled externally. The application of organic waste such as sugarcane bagasse, sugarcane molasses, wheat straw, banana skin, yam peel, saw dust, spent brewing grain, rice husk, and coconut shell into a crude oil polluted soil to detoxify the soil by the process of biostimulation (Hamoudi *et al*., 2018, Aghalibe *et al*., 2017). Agricultural waste is huge in Niger Delta area of Nigeria due to the high rainfall making agriculture predominant in the area. The output of agriculture generates high agro-waste which are becoming an environmental issue, therefore utilization of these agro waste in soil bioremediation becomes paramount. Hence the aim of this study was to assess the biodegradation of hydrocarbon in crude oil polluted soil using agricultural by-products like cassava and plantain peels with either addition of poultry droppings, cow dung or Pig slurry as inoculants for soil amendment and as nutrient enhancer.

Materials and Methods

Sample Collection and Preparation

Soil samples were collected from two (Agip Oil) spilled sites at Abacheke community Ohaji/Egbema Local Government Area of Imo State Nigeria. Four soil samples were randomly collected from the two sampling sites in each station. The soil samples were taken in depths of approximately 0-15 cm and 15-30 cm using a soil auger, they were pooled together to obtain a homogenous mixture which was divided into five different samples. Cassava peels were obtained from cassava sales unit of Ihiala environs while Cow dung, Pig slurry and Poultry droppings were collected from a farm in Ihiala town. The Cassava and Plantain peels were grinded using Manual Hand Mill Grinder, samples A, B, C and D were mixed with either Cassava or Plantain peels with the addition of Cow dung, Pig Slurry or Poultry droppings as inoculant using the ratio listed in table (1) below. The four already mixed soil samples were isolated in four different zip lock nylon labelled A-D while the fifth soil sample (without mixture sample E) was used as control. The samples were left to stay for 90 days.

Sample Code KG	Soil Weight KG	Agro-Waste KG	Inoculant KG	Percentage Ratio %
Sample A	Soil weight 2kg	Cassava peels 3kg	Pig slurry 0.5kg	Percentage ratio % 40 60
Sample B	Soil weight 1.5kg	Cassava peels 1.5kg	Poultry drooping 0.5kg	Percentage ratio % 50 50
Sample C	Soil weight 1.5kg	Cassava peels 3kg	Cow dung 0.5kg	Percentage ratio % 35 65
Sample D	Soil weight 2kg	Plantain peels 3kg	Pig slurry 0.5kg	Percentage ratio % 40 60
Sample E	Soil weight 5kg	Nill	Nill	Percentage ratio % 100

Table 1: Sample ratio for bioremediation material.

Laboratory Analysis

All the five soil samples (A-E) were analysed for Total Hydrocarbon Content (THC), Mechanical analysis for particle size distribution (Sand, Clay and Silt), Total nitrogen, Phosphorus, Organic Carbon, Exchangeable bases (Sodium, Potassium, Calcium Magnesium), Electrical conductivity, and Soil pH using the method described by Onyeonwu (2000). Aluminum ions were determined by the Colorimetric procedure described by Juo (1978) while Moisture was determined by Hot air oven method described by Ihenetu *et al.* (2017).

Result and Discussions

Result

Runs	Parameters	Sample A	Sample B	Sample C	Sample D	Sample E (Control)
1.	% Sand	93.80	91.80	91.80	93.80	93.80
2.	% Silt	2.60	3.60	2.60	2.60	2.60
3.	% Clay	3.60	4.60	3.60	3.60	3.60
4.	Texture	Sandy	Sandy	Sandy	Sandy	Sandy
5.	Total Hydrocarbon mg/kg	1282	1294	1297	1301	3637
6.	Soil pH	5.78	5.82	5.87	5.90	5.63
7.	Moisture Content %	67.50	41.20	48.70	61.90	8.76
8.	Electrical Conductivity µs/cm	1.24	1.27	1.32	1.37	1.53
9.	Organic Carbon %	1.67	1.78	2.52	2.66	1.32
10.	Phosphorus ppm	36.40	34.10	36.40	30.60	48.60
11.	Nitrogen %	0.17	0.18	0.20	0.22	0.13
12.	Calcium (Cmol. Kg^{-1})	0.020	0.0185	0.017	0.015	0.021
13.	Magnesium (Cmol. Kg^{-1})	0.083	0.066	0.048	0.035	0.1021
14.	Potassium (Cmol. Kg^{-1})	0.0041	0.0033	0.0028	0.0023	0.0048
15.	Sodium (Cmol. Kg^{-1})	0.0186	0.0152	0.0135	0.0101	0.0230
16.	Aluminum ions Al^{3+} (Cmol. Kg^{-1})	0.17	0.29	0.40	0.48	1.28

Table 2: Physico-chemical content of both untreated and treated crude oil contaminated soil samples.

Discussions

Total Hydrocarbon (THC)

Total hydrocarbon content of the soil samples is presented in (Figure. 1). Total hydrocarbon content is an index to determine the level of crude oil in the soil (Benson *et al*., 2016). The toxicity of crude oil is directly proportional to the total hydrocarbon content of the polluted site. Petroleum hydrocarbons sterilizes the soil and prevent crop growth and yield for a long period of time, the soil is soaked up by the oil like sponges and prevents the lenticels of crops to absorb oxygen causing starvation (Oyedeji, 2012). The result showed a significant reduction in THC content of the four amended soil samples when compared with the control sample (E) which had high THC of 3637mg/kg. All the organic material used in the compost significantly reduced the THC of the soil when compared to the control sample, this is in line with the findings of (Romanus *et al*., 2015, Oyodele, *et al*., 2016 and Aghalibe *et al*., 2017). The agro waste organic materials used in the bioremediation process enhanced biodegradation by supplying the required nutrients needed by the microbial community for multiplication thereby increasing the rate of degradation of hydrocarbons. Sample E (control) had high hydrocarbon content due to the low hydrocarbon reduction which occurred through natural degradation process (Romanus *et al*., 2015). Generally composting of organic waste which is aerobic process involving heat generation at the temperature range of (40°C and above), oxygen, moisture and nutrient required for the process are made available by agro waste used as substrates in the composting process or by biodegradable organisms. The hydrocarbon chains are broken down into smaller carbon compound at the same time utilizing them for their metabolic process.

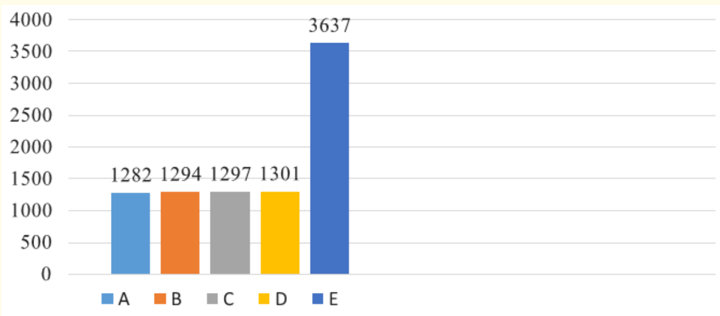

Figure 1: Bar chart showing the range of total hydrocarbon content of all the soil samples.

pH Range

Soil pH controls the biological process of the soil (Neina, 2019). In bioremediation, pH plays an important factor, most bioremediation process occurs at a pH range of nearly 5.5 to 8 which is also the approximate range at which most heterotrophic bacteria that degrade hydrocarbon perform optimally (Nazir *et al*., 2015). Sample E (Untreated polluted samples) had a pH range of 5.63 after a period of 90 days while sample D (Soil 2kg, Plantain 3kg, Pig slurry 0.5kg) had the highest pH range of 5.90 among the four contaminated treated samples this agreed with the findings of (Ekpo *et al*., 2012). However, the pH range of all the samples are within the range that is suitable for supporting efficient bioremediation process (Nazir *et al*., 2015). The pH range of the treated samples are slightly low considering the reports of (Aghalibe *et al*., 2017 and Romanus *et al*., 2015). The addition of organic manure to crude oil polluted soil increased the soil pH as was evidenced in the four treated samples. According to Ijah *et al*., (2008) organic manure has buffering effect on crude oil polluted soil because crude oil pollution has been reported to decrease soil pH. The increase in soil pH due to addition of cow dung, poultry droppings and pig slurry could have caused an increase in microbial activity during the process of decomposition and organic matter formation. This could have led to the release of more exchangeable bases that buffered pH of the soil towards neutral range (Ijah and Antai, 2003).

Moisture Content

Moisture content of the soil samples ranged from 8.76 to 67.50. Moisture is a function of physical structure of waste and ratio of air to water in the soil. During bioremediation process, when water content is too high, it will be difficult for atmospheric oxygen to penetrate the soil; this may limit the growth efficiency of organisms that are involved in the bioremediation process (Nazir *et al*., 2015). According to Shelton and Parkin (1991), the rate of biodegradation is increased when moisture content is increased. The control sample (untreated polluted samples) had a very low moisture content of 8.76% compared to the treated samples which had the moisture content that ranged from of 41.20% to 61.90%. The moisture content of the four treated samples is higher compared to 10.40-11.25% range reported by (Oyodele *et al*., 2016). The significant high moisture content of the amended soil may be from the organic agro-waste materials used as bioremediation materials; this according to Hamoudi-Belarbi *et al*. (2018) is as a result of catabolism of bacteria that are involved in bioremediation, which in turns releases high energy and water thereby increasing the moisture content of the system. Moisture content of soil could influence the oxygen availability for the biodegradation of contaminants (Lee *et al*., 2000). Samples D and A had a high amount of soil (2kg) compared to 1.5kg of other samples, this may be the reason behind the high moisture content in the two samples as against other samples.

Organic Carbon Content

Organic carbon content ranged from 1.32% to 2.66% (Table 2). Organic carbon affects soil properties such as their water holding capacity, bulk density and mobilizes nutrients for plants (Obasi *et al*., 2013). Organic carbon when present in sufficient quantity has beneficial effect on soil chemical and physical properties. It is the most abundant at the surface of the soils and sediments where detritus is deposited. Sample E (untreated polluted sample) had the least quantity of organic carbon which stood at 1.32% compared to 1.67% to 2.66% range of the treated samples. The percentage of organic carbon of the samples is slightly higher compared to the range reported by (Oyodele *et al*., 2016) whereas it agreed with the range reported by (Ekpo *et al*., 2012). There was an increase in the organic carbon of the treated soil samples compared to the control sample; this may be from the addition of agro waste materials like animal droppings and food waste which released organic carbon during decomposing thereby enhancing the organic carbon content of the four amended samples. Again addition of agro waste materials enhanced biodegradation of hydrocarbons in the four treated soil samples breaking the carbon chain into smaller sediments thereby increasing the organic content of the four samples against the control (Okolo *et al*., 2005).

Electrical Conductivity

Electrical conductivity (EC) is the current flow in the soil which is proportional to the total dissolved solids in the soil (Othaman *et al*., 2020). It is the measure of the amount of salts in soil which is an important indicator of soil health. Soil electrical conductivity is the measure that correlates with the soil properties that affect crop productivity, including soil texture, cation exchange capacity, drainage condition, organic content, salinity and subsoil characteristics (Devatha *et al*., 2019). The EC of the untreated soil sample (E) (1.53 μs/cm) which was significantly higher than the four amended soil samples (1.24μs/cm to 1.37μs/cm). Devatha et al. (2019) reported that soil texture is one of the factors that affects EC, the soil samples under consideration were reported to be sandy which according to Othaman *et al*. (2020) sandy soil does not hold moisture very well due to its surface contact degree hence they normally have low electrical conductivity.

Nitrogen

Nitrogen exists in the soil system in many forms and changes very easily from one form to another (Lamb et al., 2014). It is added to the soil naturally through nitrogen fixation by soil bacteria and legumes through atmospheric deposition during rainfall. Nitrogen is the most important plant nutrient for crop production since high amount of nitrogen are required compared to other essential nutrients (Hofman and Cleemput, 2004). Aside from natural nitrogen cycle through fixation, nitrogen is also found in organic materials (plant residues, animal manures, sewage, and soil organic matter) they are present as part of protein, amino acids plus other plant and microbial material. The process, which is completed by soil microbes as a product of organic matter decomposition through a process called mineralization (Crohn, 2004). Sample E had 0.13% nitrogen availability while that of the treated samples ranged between 0.18% to 0.22%which agreed with the findings of (Oyodele *et al*., 2016 and Ekpo *et al*., 2012). The treated samples witnessed slight improvement in their nitrogen content compared to the untreated control sample E which agreed with the findings of (Aghalibe *et al*., 2017, Offiong, *et al*., 2021). Sample D had the highest nitrogen range of 0.22% which is higher compared to other treated samples this may be from the nutrient released by plantain peels and Pig slurry which enhanced microbial activities (Jude *et al*., 2019). Oyodele *et al*. (2016) reported that the treatment of crude oil polluted soil with maize husk compost increased the nitrogen content compared to the untreated soil sample.

Phosphorus

Phosphorus ranged from 30.60ppm to 48.60ppm. Phosphorus is one of the most essential nutrient elements for plant growth and development. Phosphorus plays an important role in maintaining soil fertility and makes the agricultural production system more sustainable (Wu *et al*., 2020). It is the second most important macronutrient and an essential plant nutrient (Srinivasan, *et al*., 2012). When soil is deficient in phosphorus, it limits plant growth (Muindi, 2019). In soil, phosphorus exists in various chemical forms including inorganic and organic form. When organic phosphorus is not sufficient in the soil, it is supplied by treating the soil with compost involving decomposing plant and animal products, along with the soil microflora and fauna through mineralization process (Muindi, 2019). In this context, sample E (untreated control sample) had the highest phosphorus level of 48.20ppm as against the range of 30.60ppm to 36.40ppm recorded by other four treated samples. The untreated sample had the highest level of phosphorus due to less activity of microorganisms which have been found to utilize phosphorus as food during the mineralization process that degraded the crude oil (Benson *et al*., 2016). Sample D (Soil 2kg, Plain peels 3kg, Pig slurry 0.5kg) had the lowest phosphorus content of 30.6ppm among the four amended samples due its high organic carbon content which might have influenced the microbial activity; these organisms might have utilized the phosphorus during the decomposing process. The reduction in the range of phosphorus in all the treated samples is in accordance with the findings of Benson *et al*. (2016) and Devatha *et al*. (2019).

Exchangeable Cations

Calcium, magnesium, potassium and sodium are charged ions usually absorbed by electrostatics or columbic attraction to the soil surface colloids (Okon and Ogba, 2018). Ogeleka *et al*. (2017) ascertained that exchangeable cations affect the physical properties of the soil and soil fertility level connecting soil acidity and basicity. The exchangeable cations under consideration were high in sample E (untreated sample) due to the chemical impact of crude oil contamination; however, they were slightly reduced after the bioremediation process. The range of Ca, Mg, K and Na in the entire soil sample (A-E) is low compared to the report of (Oyodele *et al*., 2016). Their low exchangeable cation range may be from the soil texture which was found to be sandy. Sandy soil has been reported to be deficient in soil mineral due to its high pH level, nutrient cations (Ca, Mg, Na and K) are attracted to negative exchange site with the pH range of 3 (Ogeleka *et al*., 2017). Al^{3+} content of the four treated sample plus the control showed that control sample E had 1.28Cmol/Kg which is higher compared to the range of 0.48 Cmol/Kg, 0.40 Cmol/Kg, 0.29 Cmol/Kg, 0.17 Cmol/Kg recorded by sample D, C, B and A respectively. The range was slightly lower compared with the report of (Ekpo *et al*., 2012). Ekpo *et al*. (2012) and Lauricella *et al*. (2020) reported a decrease in Al^{3+} content of crude oil contaminated soil after its amendment with organic remediating materials, the reduction he said was not directly connected to decrease in pH.

Soil Particle Size

The soil particle size wasn't affected by remediation treatment except for percentage of sandy soil in sample B (Soil 1.5kg, Cassava 1.5kg, Poultry dropping 0.5kg) and C (Soil 1.5kg, Cassava 3kg, Cow dung 0.5kg) treatment which agreed with the findings of (Oyodele *et al*., 2016) that amendment of soil didn't change the particle size of the soil samples. The slight difference in the soil particle size in sample B and C may be from the lesser quantity of soil used (1.5kg) in sample B and C compared to (2kg) used in other treatment samples. The percentage of silt and clay in the entire soil samples (A-E) were the same except for sample B (with 0.5kg poultry droppings added in the sample mixture) which had 3.60% silt and 4.60% clay against 2.60% silt and 3.60% clay in other samples. This slight increase might have been from organic activities supported by addition of poultry dropping which have been found to improve soil quality (Dikinya and Mufwanzala, 2010).

Conclusion

The successful conduct of this research revealed that bioremediation of crude oil contaminated soil using organic by-products like cassava and plantain peels together with cow dung, poultry droppings and pig slurry is possible and will conserve foreign reserves for low and mid-income countries like Nigeria. From the findings, the four different soil treatment options reduced the total hydrocarbon content of the crude oil polluted soil samples when compared to the untreated sample. Therefore, it is highly recommended that these affordable organic agro-waste materials should be freely employed by oil companies especially in Niger Delta area of Nigeria that are currently battling with the adverse impact of crude oil contamination which has affected both agriculture, health and general livelihood of the people.

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Conflict of Interest

The authors declare there is no conflict of interests.

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