

STUDIES IN THE SUITABILITY OF NPK-15-15-15 FERTILIZER, ANIMAL DUNG, AND PETROLEUM AGAR AS NUTRIENT SUPPLEMENTS FOR GROWTH OF MICROORGANISMS

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ABSTRACT

The suitability of three nutrient supplements (NPK-15-15-15 fertilizer, petroleum agar, and animal (cattle) dung) in promoting growth of microorganisms in produced water and crude-oil-contaminated soil are investigated experimentally. The analysis involves monitoring the total microbial count (TMC) in produced water and crude-oil-contaminated soil samples treated with the different nutrient supplements. After a test period of 10 weeks, the TMC in produced water treated with NPK, petroleum agar, and cattle dung was 10^5 cfu/ml, 10^3 cfu/ml, and 10^2 cfu/ml respectively; while the TMC in soil treated with NPK, petroleum agar, and cattle dung was 10^4 cfu/g, 10 cfu/g, and 10^2 cfu/g respectively. NPK-15-15-15 fertilizer exhibits the best characteristics as nutrient supplement for the microorganisms in both the produced water and crude-oil-contaminated soil; followed by petroleum agar and then cattle dung for produced water, but followed by cattle dung and then petroleum agar for crude-oil-contaminated soil.

KEYWORDS: NPK fertilizer; Petroleum agar; Cattle dung; Microbes; Nutrient.

1. INTRODUCTION

Contamination of the biophysical environment by pollutants and the attendant potential effects on human and the ecosystems is a problem of global concern. For example, various environmental effects of oil exploration and exploitation such as killing of marine organisms and damage to plants; making water unsafe for drinking, recreational activities, and domestic purposes; and destruction of natural components of soil are reported in literature (Joseph and others, 1996; Ojo, 1998). Environmental regulations require that contaminated area be cleaned to an acceptable and permissible level (DPR, 2002). Clean-up techniques which may be physical, chemical, or biological, are always applied to oil-degraded environment to restore its productivity. Physical clean-up methods include direct disposal of wastes/debris, while chemical methods involve the use of chemicals substances such as dispersants and surfactants. Although physical and chemical clean-up methods are most widely used, they are not cost effective and sometimes result in further contamination of an environment (Stevens, 1991).

Biological clean-up methods involve the use of biological agents such as microorganisms and such approaches are environmentally friendly (Leahy and Colwell, 1990). Applications of microorganisms and nutrients in bioremediation of crude-oil-contaminated soils are reported in literature (Osonubi and others, 1991; Adenipekun and Fasidi, 2005; Tisdall, 1991). The success of microorganisms in the degradation of pollutants in any environment depends solely on the substrate for the microorganisms and the physico-chemical properties of the environmental (Anderson and Domsch, 1975). Nutrient supplements such as poultry manure, animal (cattle) dung, urea fertilizer, NPK-15-15-15 fertilizer, and oyster mushroom (*lentinus subnudus*), are used to stimulate biodegradation of contaminants on land and water (Adenipekun and Fasidi, 2005; Rim-Rukeh, 2005; Kuforiji and others, 2003; Adieze and others, 2003; Ehigiator, 2003). In this study, we compare the suitability of inorganic fertilizer (NPK-15-15-15), petroleum agar, and animal (cattle) dung as nutrient supplements (substrates) for the growth of microorganisms in produced water and crude-oil-contaminated soil. The choice of these nutrients supplements is based on their reported

environmental friendliness and application in bioremediation of crude-oil-contaminated environment (Rim-Rukeh, 2005; Adieze and others, 2003; Lee and others, 1993).

2. MATERIALS AND METHODS

The materials used in the study are crude-oil-contaminated soil, produced water, NPK-15-15-15 inorganic fertilizer, petroleum agar, animal (cattle) dung, four (4) beakers, one (1) plastic bucket, and three wooden boxes measuring 30cm × 30cm × 10cm. The petroleum agar used as nutrient supplement in the experiments was procured from NEK Chemicals (Nig.) Ltd, Port Harcourt, and manufactured by BCH Chemicals, London; it contains 0.5g of ammonium chloride (NH₄Cl), 2.5g of hydrated sodium hydrophosphate (NaHPO₄12H₂O), 5g of potassium hydrophosphate (K₂HPO₄), and 15g of Diefo agar powder, per litre of engine oil.

The animal (cattle) dung used in the study was obtained from abattoir located at Slaughter market in Trans-Amadi Industriail Layout, Port Harcourt, Rivers State, Nigeria. The animal (cattle) dung is reported to contain 0.53-0.85%N, 0.25-0.42%P, and 1.31-2.22% K (Cooke, 1992). The inorganic fertilizer (NPK-15-15-15) used in the study was manufactured by NAFCON (National Fertilizer Company of Nigeria) in 1999 and contains 0.15g of nitrogen, 0.065g of phosphorus, and 0.125g of potassium.

The crude-oil-contaminated soil used in the study was collected at Ebocha well 8 Oil Location (143242 Northing and 474506 Easting) in Ogba/Egbema/Ndoni Local Government Area (ONELGA) of Rivers State, Nigeria. The area was accidentally contaminated in June 2006 due to crude oil spill (NAOC, 2006), and the soil sample used in the study was collected in December 2006. (Note that collecting contaminated soil sample about six months after the spill is advantageous since the population of microorganisms in the soil would increase with time due to availability of nutrient (crude oil)). About 6kg of the contaminated top-soil was collected within 10cm depth using a hand trowel at different spots of the contaminated area. The uncontaminated soil has been reported to contain four groups of microorganisms: (i) Hydrocarbon Utilizing Bacteria (HUB) such as *Pseudomonas* sp. and *Bacillus* sp.; (ii) Heterotropic Bacteria (HB) such as

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Pseudomonas sp., *Bacillus* sp. and *Norcadia* sp.; (iii) Hydrocarbon Utilizing Fungi (HUF) such *Saccharomyces* sp., *Penicillium* sp., and *Candida* sp.; and (iv) Heterotrophic Fungi (HF) such as *Saccharomyces* sp., *Penicillium* sp. and *Candida* sp., which are also present in the contaminated soil (EIA, 2006): Total microbial count in the contaminated soil lies in a range from 1.52×10^3 to 8.6×10^4 cfu/g for bacteria, and a range from 9.0×10^1 to 1.87×10^3 cfu/g for fungi (EIA, 2006). The contaminated soil was placed in a clean plastic bucket and taken to the laboratory for analysis.

Three (3) litres of produced water used in the study was obtained from a gas plant in Rivers State, Nigeria. The produced water sample was collected in a plastic container that has been sterilized by washing with dilute hydrochloric acid and rinsed with distilled water. At the sample collection point, the container was first of all rinsed twice with the produced water, and then filled to the brim and tightly capped. The produced water sample contains the same four groups of microorganisms as the contaminated soil of Ebocha well 8 Oil Location (EIA, 2005). Total microbial count in the produced water lies in a range from 1.71×10^1 to 5.07×10^2 cfu/ml for bacteria, and a range from 6.10×10^1 to 2.2×10^3 cfu/ml for fungi (EIA, 2005). The produced water sample was taken to the laboratory for analysis.

2.1. Experimental procedures

(i) Produced water

Three (3) litres of the produced water sample collected for the study was divided equally into three (3) beakers labeled according to the different nutrient supplements. Two grammes (2g) of NPK-15-15-15 fertilizer was added to beaker 1 to serve as a source of nutrient for the microorganisms in the produced water in this beaker. The same quantity of petroleum agar and animal (cattle) dung was added to the produced water in beakers 2 and 3 respectively, and the experimental set-up was left for a period of ten (10) weeks. Total microbial count (TMC) in each beaker was determined at the start of the experiment and at intervals of 2 weeks (i.e. 0, 2, 4, 6, 8, and 10 weeks) throughout the test period. TMC was determined using the rapid agar dip-stick technique through a calibration chart

provided by the manufacturer (Boots Micro – check company, Nottingham, UK).

(ii) Crude-oil-contaminated soil

The non-in-situ remediation method of Nyborg and McGill (1975) was adopted. Three (3) wooden boxes labeled according to the different nutrient supplements and measuring 30cm by 30cm by 10cm were constructed and lined with perforated polyethylene bags. Two kilogrammes (2kg) of crude-oil-contaminated soil was measured into each of the wooden boxes and the soil in each box was dosed with the same volume (3 litres) of the corresponding nutrient supplement (i.e. NPK-15-15-15 solution obtained by dissolving 3g of the fertilizer in 3 litres of water, petroleum agar medium obtained by dissolving 3g of petroleum agar in 3 litres of engine oil, and cattle dung slurry obtained by dissolving 3g of cattle dung in 3 litres of water). Each box containing the soil sample and the nutrient supplement was tilled to properly mix the nutrient supplement and the soil, and the experimental set-up was left for a period of 10 weeks. Total microbial count (TMC) in each wooden box was determined (using the rapid agar dip-stick technique) at the start of the experiment and at intervals of 2 weeks (i.e. 0, 2, 4, 6, 8, and 10 weeks) throughout the test period. The experimental procedures described above are consistent with DPR (2002) and APHA (1990) standards.

3. RESULTS AND DISCUSSION

Figure 1 shows the effects of the three nutrient supplements (NPK, petroleum agar, and cattle dung) on the total microbial population in the produced water sample. (Since TMC values are presented in powers of 10, a semi-log plot is chosen to indicate only the powers of 10 on the TMC-axis). It may be seen from Fig. 1 that the TMC in the three beakers are the same (10^3 cfu/ml) at the start of the experiment. Thereafter, the TMC in beaker 1 (containing NPK-15-15-15) increases tremendously from 10^3 to a maximum value of 10^8 cfu/ml within six weeks of the experiment and decreased from 10^8 to 10^5 cfu/ml within the last four weeks. In beaker 2 (containing petroleum agar), there was increase in TMC from 10^3 to a maximum value of 10^6 cfu/ml within four weeks of the experiment which was maintained until the 6th week, and then decreased from 10^6 to 10^3 cfu/ml within the last four weeks.

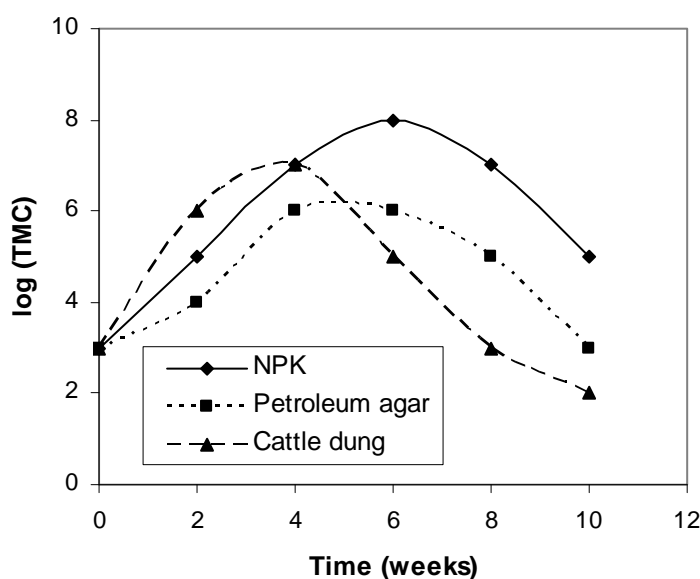


Fig. 1: Effects of NPK, petroleum agar, and cattle dung on TMC in produced water.

In beaker 3 (containing cattle dung), the TMC increases from 10^3 to a maximum value of 10^7 cfu/ml within four weeks of the experiment and decreased from 10^7 to 10^2 cfu/ml within the last six weeks. At the end of the test period, the population of microorganisms in the beaker with NPK is higher than in the beaker with petroleum agar, with the least microbial population in the beaker containing cattle dung. Although cattle dung results in higher microbial growth than NPK and petroleum agar in less than four weeks of the experiment, it could not sustain growth of the microorganisms beyond the fourth week. Since long-term growth and sustenance of microorganisms is required in bioremediation processes, NPK 15-15-15 fertilizer exhibits the best characteristics as nutrient supplement for the microorganisms in the produced water, followed by petroleum

agar, with cattle dung as the least effective nutrient supplement.

Figure 2 shows the effects of the three nutrient supplements on the total microbial count in the crude-oil-contaminated soil sample. The TMC in the three boxes are the same (10^4 cfu/g) at the start of the experiment. Thereafter, the TMC in box 1 (containing NPK15-15-15) increases tremendously from 10^4 to a maximum value of 10^9 cfu/g within six weeks of the experiment and then decreased from 10^9 to 10^4 cfu/g within the last four weeks. In box 2 (containing petroleum agar), there was increase in TMC from 10^4 to a maximum value of 10^6 cfu/g within four weeks of the experiment and decreased from 10^6 to 10^1 cfu/g within the last six weeks.

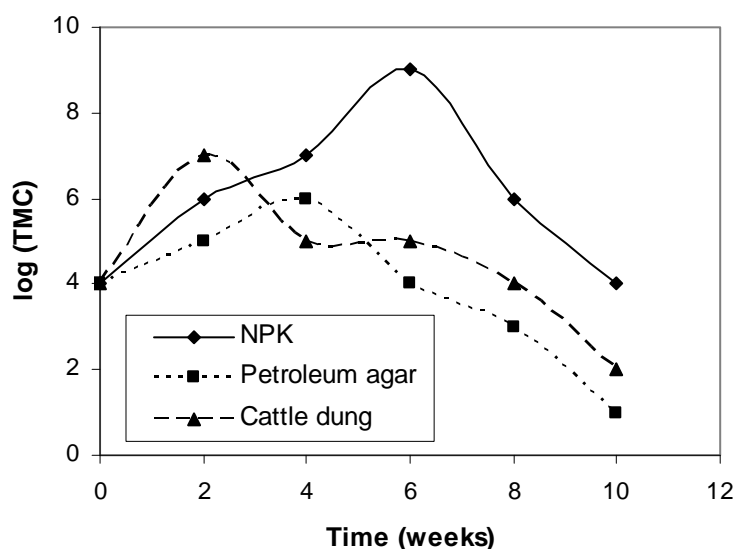


Fig.2. Effects of NPK, petroleum agar, and cattle dung on TMC in crude-oil-contaminated soil.

In box 3 (containing cattle dung), there was increase in TMC from 10^4 to a maximum value of 10^7 cfu/g within two weeks of the experiment and decreased from 10^7 to 10^2 cfu/g within the last eight weeks. At the end of the test period, the population of microorganisms in the box with NPK is higher than in the box with cattle dung, with the least microbial population in the box containing petroleum agar. Although cattle dung still results in higher microbial growth in the soil sample than NPK and petroleum agar within two weeks of the experiment, it could not sustain growth of the microorganisms beyond the second week. Here, NPK again exhibits the best characteristics as nutrient supplement for the microorganisms in the crude-oil-contaminated soil; followed by cattle dung, with petroleum agar as the least effective nutrient supplement in this case. The increase in TMC in Figs. 1 and 2 may be attributed to the supply of nutrients by the nutrient supplements, while the reduction in TMC is a result of depletion of nutrients in the media (Adenipekun and Fasidi, 2005; Rim-Rukeh, 2005; Kuforiji and others, 2003; Adieze and others, 2003; Ehigior, 2003; Lee and others, 1993).

4. CONCLUSION

Laboratory investigations on the suitability of NPK-15-15-15 fertilizer, petroleum agar, and animal (cattle) dung as nutrient supplements for the growth of microorganisms in produced water and crude oil contaminated soil are presented. It is shown that NPK-15-15-15 fertilizer exhibits the best characteristics as nutrient supplement for the microorganisms in both the produced water and crude-oil-contaminated soil;

followed by petroleum agar and then cattle dung for produced water, but followed by cattle dung and then petroleum agar for crude-oil-contaminated soil.

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