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Performance of Organic and Inorganic Substrates for the Bioremediation of Crude Petroleum Hydrocarbon Polluted Soil

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Abstract

This paper presents an evaluation of the performance of dead cells microorganism such as yeast, organic substrate such as poultry manure, decayed domestic waste manure and inorganic substrate such as N.P.K fertilizer in the bioremediation of petroleum hydrocarbon polluted soil. Hydrocarbon polluted soil was simulated on a laboratory scale and characterized to determine the particle size distribution, pH, moisture content, total organic carbon, total nitrogen and total hydrocarbon content. For effective treatment, 2:1 mixture (w/w) of the polluted soil and the treatment substrate in addition to 25ml of nutrient agar was utilized. The entire experiment was monitored for a period of twelve (12) weeks and triplicate samples were collected from each setup on a weekly basis and analyzed using ultra violet spectrophotometer in order to determine the residual hydrocarbon content. The amount of hydrocarbon removed per time coupled with the efficiency of remediation was then computed using simple mass balance equation. Results obtained revealed a gradual decrease in the total nitrogen content (TNC), total organic carbon (TOC) and total hydrocarbon content (THC) with remediation time. This decrease in TNC, TOC and THC was also observed to vary with the type of substrate as follows. For yeast; TOC reduces from 2.169 to 0.16g/kg, TNC reduces from 5.20 to 1.53g/kg, and THC reduces from 11.533 to 3.18mg/l. For poultry manure; TOC reduces from 2.169 to 0.14g/kg, TNC reduces from 5.20 to 1.76g/kg, and THC reduces from 11.533 to 3.54mg/l. For NPK; TOC reduces from 2.169 to 0.11g/kg, TNC reduces from 5.20 to 2.43g/kg, and THC reduces from 11.533 to 2.02mg/l. For decomposed waste; TOC reduces from 2.169 to 0.98g/kg, TNC reduces from 5.20 to 2.08g/kg, and THC reduces from 11.533 to 9.17mg/l. The gradual decrease in TNC, TOC and THC was attributed to the fact that; as the microorganism utilizes the available nitrogen and organic carbon present in the soil and increase in population; they again react with and break down the petroleum hydrocarbon.

1. Introduction

Continuous production, movement, and distribution of petroleum and its components during the last century have resulted in occasional problems of hydrocarbon-contamination of land and water due to spillage resulting in a major environmental problem and cause serious damage to the environment [1, 2]. Petroleum hydrocarbon pollution has contaminated waters, threatened human health and causes damage to the environment. Hydrocarbons have leaked from tankers into the oceans and from underground storage tanks into soils and ground waters. Although it is the large marine oil spills with the pictures of dead seabirds that attract public attention, most environmental hydrocarbon contaminants originate from much smaller leakages, such as improper disposal of waste motor oils and leaking underground storage [3, 4]. The cost implication of reclaiming the land

and cleaning the water bodies after every incidence of contamination resulting from spillage has remained a major problem to the developing Nations, Nigeria inclusive [5, 6]. Before now, chemical cleaning has remained the most practice option to tackle the resultant effects of hydrocarbon pollution. Apart from the cost and complexity that is associated with this method, there is also the problem of secondary toxic end product after every chemical cleaning operation [7, 8, and 4].

Bioremediation, (involving the use of microorganism from organic/inorganic substrate (Bio-augmentation) or green plants and their associated microorganism (Phyto-remediation), offers possible solution to this problem. Bioremediation extends the natural processes by which microorganisms consume organic molecules, including hydrocarbons [9, 10, and 11]. The micro-organisms convert organic molecules to cell biomass and products such as carbon dioxide and water that can be readily accommodated in the environment. Bioremediation of petroleum pollutants aims to increase the natural rates of hydrocarbon biodegradation that produces non-toxic end products. One of the basic limitations to this practice of using isolated living cells microorganism is the problem of disposal. Microorganism if not properly disposed can cause serious outbreak of disease. On account of this, living cells microorganism are gradually paving way for dead cells microorganism which are easier to handle and control [12]. It has been known for 80 years that certain microorganisms are able to degrade petroleum hydrocarbons and use them as a sole source of carbon and energy for growth.

Petroleum hydrocarbon (PHC) contamination of soil is a concern for a number of reasons. First of all, once released into soil, the volatility of PHC can pose a fire or even explosion hazard especially when vapour enters confined spaces. Secondly, contaminants can interfere with the nutrients and water transmission and thus lead to land degradation. Thirdly, weathered petroleum residuals may stay bound to soil particles and be retained in soil for years. Fourthly, although these contaminants may benefit the oil degraders as a carbon source, they are still toxic to the majority of soil biota [13]. PHC pollutants can strongly alter the ecology and the physiology of bacteria and fungi [14]. Fifthly, PHCs may destroy the aesthetic by inducing offensive odour, taste or appearance in environmental media. Last but not least, PHC contamination of soil is not only a concern for the soil itself, but is also a potential danger to the plant population.

2.0 Experimental Procedure

2.1 Samples Collection

The soil sample used for this research was collected from the top surface soil (0-15 cm) in the Faculty of Engineering, behind Petroleum Engineering building, University of Benin, Benin City; using a hand auger. Dead cell microorganism (yeast) was gotten from a pharmaceutical store, organic substrates (poultry manure and decayed waste manure) were gotten from Uniben integrated farm project while the inorganic substrate (NPK manure) was purchased from a chemical store. The crude petroleum hydrocarbon was obtained from Warri Refinery and Petrochemical Company (NNPC).

2.2 Soil sample preparation, simulation and amendment

The sample soil was air dried for approximately one week, homogenized and passed through 2mm pore size sieve and was stored in black polyethylene bag prior to use. 2:1 mixture of the soil and the crude oil was obtained in a plastic buckets. The mixture was properly mixed, covered with a layer of aluminum foil paper and left for four days to stabilize before commencement of treatment. For effective treatment, a 2:1 mixture (w/w) of the polluted soil and the substrate in addition to 25ml of

nutrient agar was utilized [15]. The plastic container and its content were open throughout the period of experimentation to allow for the influence of atmospheric oxygen.

2.3 Soil characterization/ physicochemical analysis

Before contamination, the soil sample was characterized after digestion (using 1:1 ratio of 0.25M hydrochloric acid and Nitric acid) to determine the intrinsic properties of the soil. Some of the properties of interest include [16].: total heterotrophic bacterial (THB), pH, electrical conductivity, total hydrocarbon content (THC), total, organic carbon (TOC), total phosphorous (TP), total nitrogen (TN). After contamination, the hydrocarbon polluted soil sample was again subjected to chemical digestion using 1:1 ratio of 0.25M hydrochloric acid and nitric acid. Thereafter, the polluted soil was characterized to determine the same physicochemical properties enumerated as above.

2.4 Experimental setup

Five experimental set-ups were utilized for the study as follows; to the first bucket, a 2:1 mixture of the polluted soil and dead yeast with 25ml of nutrient agar was obtained for further investigation. To the second bucket, a 2:1 mixture of the polluted soil and inorganic fertilizer with 25ml of nutrient agar was obtained for further investigation. To the third bucket, a 2:1 mixture of the polluted soil and poultry manure with 25ml of nutrient agar was obtained for further investigation. To the fourth bucket, a 2:1 mixture of the polluted soil and organic manure from decayed domestic waste material with 25ml of nutrient agar was obtained for further investigation and the fifth bucket was used as the control set up containing the polluted soil with no treatment substrate and nutrient agar added.

2.5 Analysis of treated soil samples

The entire experiment lasted for a period of twelve (12) weeks. Single sample were collected for analysis on weekly basis and the sample mean and standard deviation was computed to ascertain the rate of hydrocarbon degradation with treatment time. Analysis of the treated soil was aimed at evaluating the overall effects of treatment on the initial conditions of the soil after pollution. As stated above, physicochemical properties such as total heterotrophic bacterial (THB), pH, electrical conductivity, total hydrocarbon content (THC), total, organic carbon (TOC), total phosphorous (TP), total nitrogen (TN) were evaluated weekly.

2.5 Efficiency of remediation

The amount of hydrocarbon removed during the series of batch investigation was determined using the mass balance equation of the form

$$q = \frac{v}{m}\left[C_0 - C_e\right] \tag{1}$$

Where: q, defines the hydrocarbon uptake (mg/g); C_0 and C_e: are the initial and equilibrium hydrocarbon concentrations in the digested soil solution [mg/l] respectively; V: is the weight of soil sample taken (g) and M: is the mass of substrate used (g).

The efficiency of hydrocarbon removal (%) was calculated using the mass balance equation of the form

Removal Efficiency (%) = $\left(\frac{C_0 - C_e}{C_0} \times 100 \right)$ (2)

Where: C_0 and C_e are total hydrocarbon content (THC) (mg/l) in digested soil solution before and after treatment respectively.

3.0 Results and Discussion

Result of winsieve classification of the experimental soil is presented in Table 1 while the physico-chemical properties is presented in Table 2

Table 1: Percentage compositions of experimental soil

Parameters	Values
% Clay	7.20
% Silt	72.8
% Fine Sand	9.00
% Medium Sand	11.0
% Coarse Sand	0.00
% Fine Gravel	0.00
% Coarse Gravel	0.00
% Cobbles	0.00

The indication is that, the experimental soil contains very high composition of silt as seen in Table 1. This result justifies the level of carbon and nitrogen found in the soil before the hydrocarbon pollution. Again it also suggested that a reasonable level of microorganism will be present in the soil that will help aid the process of hydrocarbon degradation.

Table 2: Physico-chemical properties of the experimental soil

S/N	Parameter	Mean Test Result of unpolluted soil	Mean Test Result of polluted soil
1	Moisture Content (%)	1.5	25.41
2	pH	6.9	8.03

3	Electrical Conductivity (µs/cm)	59.83	230
4	Total Dissolved Solids (TDS)	33.67	151.8
5	Organic Carbon (g/kg)	16.457	2.16
6	Total Nitrogen (g/kg)	9.023	5.20
7	Total Hydrocarbon Content (mg/kg)	0.00	11.533

Result of Table 2 revealed that, addition of hydrocarbon to soil alters the initial properties of the soil as follows; the soil became highly alkaline with a mean pH of 8.03, high level of conductivity occasioned by the presence of high concentration of dissolved solids as seen from the mean results of conductivity and total dissolved solids (230µS/cm and 151.8mg/l) with a drastic reduction in total nitrogen and total carbon concentration caused by a slight increase in the soil petroleum hydrocarbon content due to pollution.

The experiment involving the bioremediation of crude petroleum hydrocarbon polluted soil commenced after four days of stabilization and was monitored for a period of twelve (12) weeks using inorganic substrate (NPK) and organic substrate (yeast, poultry manure, and decomposed waste). Triplicate samples were taken on weekly bases and the mean results were employed to evaluate the effect of treatment time and the progress of remediation. Figure 1 shows the effect of treatment time and the performance of the various substrates in the bioremediation process with reference to variation in the total nitrogen content (TNC).

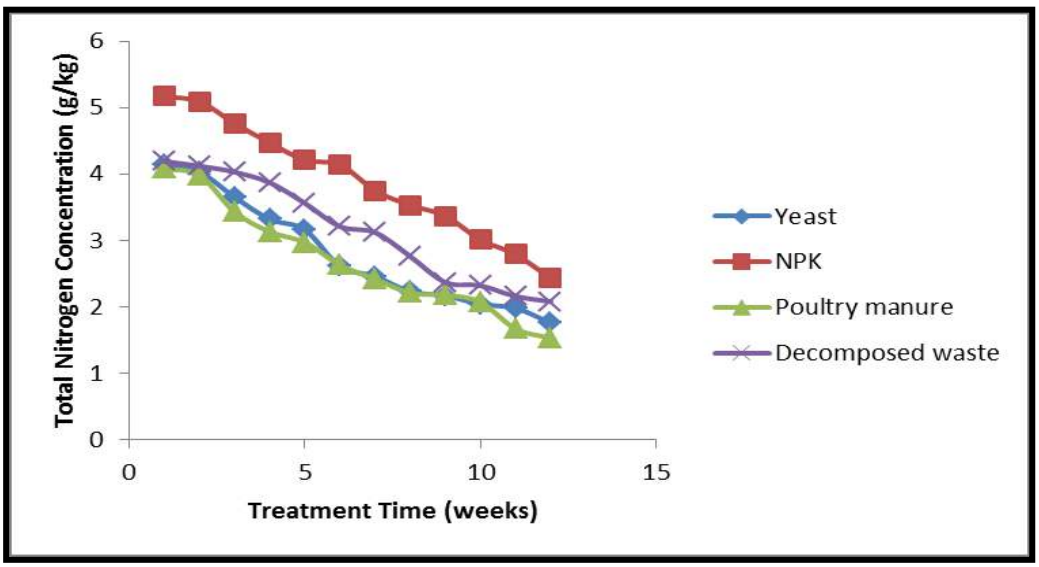

Figure 1: Variation of total nitrogen with treatment time

From the results of Figure 1, it was seen that, there was a gradual decrease in the total nitrogen content (TNC) throughout the entire period of experimentation. For substrate A; (yeast), the total nitrogen content (TNC) reduces from the initial 5.20 g/kg to 1.76 g/kg, for substrate B; (NPK), the

total nitrogen content (TNC) reduces from the initial 5.20 g/kg to 2.43 g/kg, for substrate C; (poultry manure), the total nitrogen content (TNC) reduces from the initial 5.20 g/kg to 1.53 g/kg, and for substrate D; (decomposed waste) the total nitrogen content (TNC) reduces from the initial 5.20 g/kg to 2.08 g/kg. The reduction in the total nitrogen content observed during the period of remediation could be attributed to the gradual utilization of this nutrient by the microorganism for growth and development. Again the concept of adaptability and survival of the fittest will come in to play and bring about a gradual decrease in the total nitrogen concentration since the microorganism will tend to struggle for the available nutrient to stay alive.

Results of the effect of treatment time and the performance of the various substrate in the bioremediation process with reference to variation in total organic carbon (TOC) is presented in Figure 2.

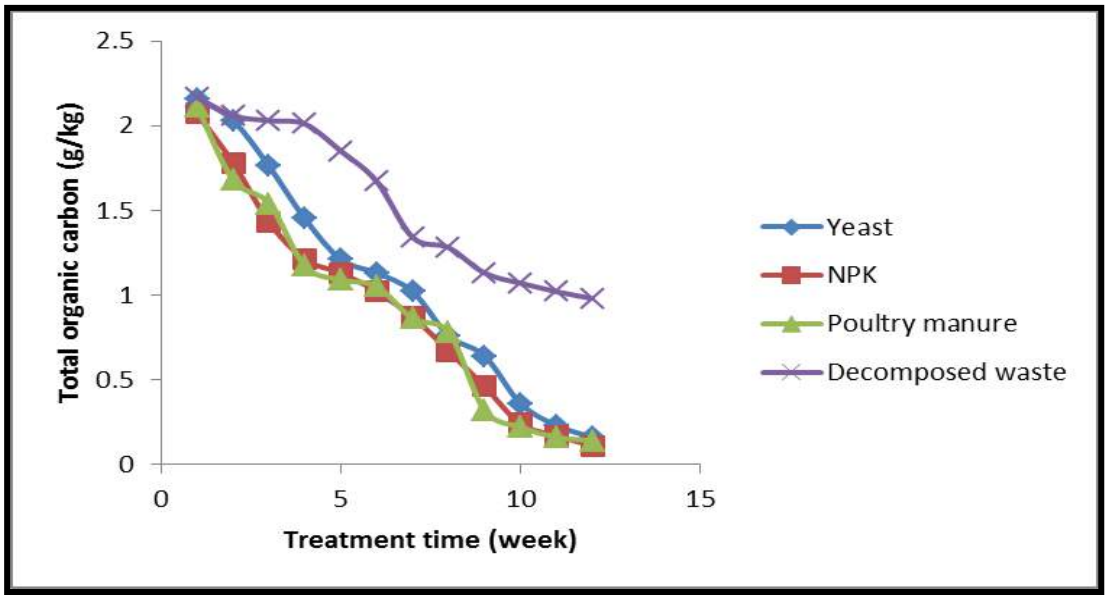

Figure 2: Variation of total organic carbon with treatment time

It was observed from the result Figure 2, that, there was a gradual decrease in the total organic carbon (TOC) throughout the entire period of experimentation. This trend is expected since the nutrient level of soil is normally expressed in terms of the concentration of nitrogen, carbon and phosphorous which the microorganism will need for their growth and cell development. Analysis of the result shows that; for substrate A; (yeast), total organic carbon reduces from the initial 2.169 g/kg to 0.16 g/kg after 12 weeks of remediation, for substrate B; (NPK), total organic carbon reduces from the initial 2.169 g/kg to 0.11 g/kg after 12 weeks of remediation, for substrate C; (poultry manure), total organic carbon reduces from the initial 2.169 g/kg to 0.14 g/kg after 12 weeks of remediation and for substrate D; (decomposed waste) total organic carbon reduces from the initial 2.169 g/kg to 0.98 g/kg after 12 weeks of remediation. Like nitrogen, there is also a gradual reduction in the total organic content (TOC) throughout the period of experimentation. This reduction can also be attributed to the gradual utilization of this nutrient by the microorganism for growth and development.

For total hydrocarbon content (THC) as observed in Figure 3, a gradual reduction was seen throughout the period of the experiment.

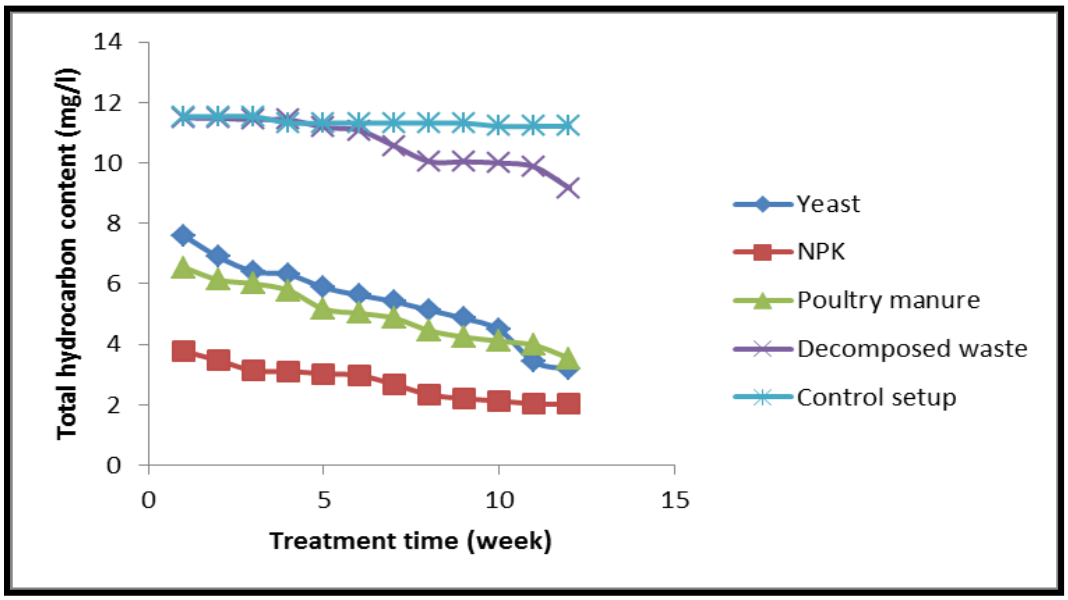

Figure 3: Variation of total hydrocarbon content with treatment time

It was observed during the entire period of experimentation that the total hydrocarbon content (THC) decreases gradually for all the substrate used due to degradation by the microorganism present in the soil. As the microorganism utilizes the available nitrogen and organic carbon nutrient present in the soil and increases their population, they again react and break down the agent that causes the pollution which is the petroleum hydrocarbon. The breakdown of petroleum hydrocarbon occasioned by increase in the population of the soil microorganism consequently results to a gradual decrease in petroleum hydrocarbon concentration as shown in Figure 3. Result of the control setup shows that, there was no remediation since the microorganism do not have supplementary nutrient to resist the effect of hydrocarbon pollution. The breakdown of petroleum hydrocarbon by the available microorganism results in the formation of carbonzylic acid as by product which in turn will have effect on the pH of the system as observed in Figure 4.

It was observed that for substrate (A: yeast), there was an initial drop in the pH for the first week of remediation up to 5.7 due to the breakdown of THC to produce carbonzylic acid. Thereafter, the pH begins to increase with time and stabilizes at 7.3 to 7.4 from week 7 to week 12. For substrate (B: NPK) a drop in pH was also observed in the first week (6.4) before it stabilizes to 7.6 in the last four weeks of remediation. For substrate (C: poultry manure), a drop in pH from 6.6 to 7.5 was observed between week one and week twelve respectively. On the whole, an initial reduction in pH value was observed for all the substrate used during the period of experimentation due to breakdown of hydrocarbon to acid components.

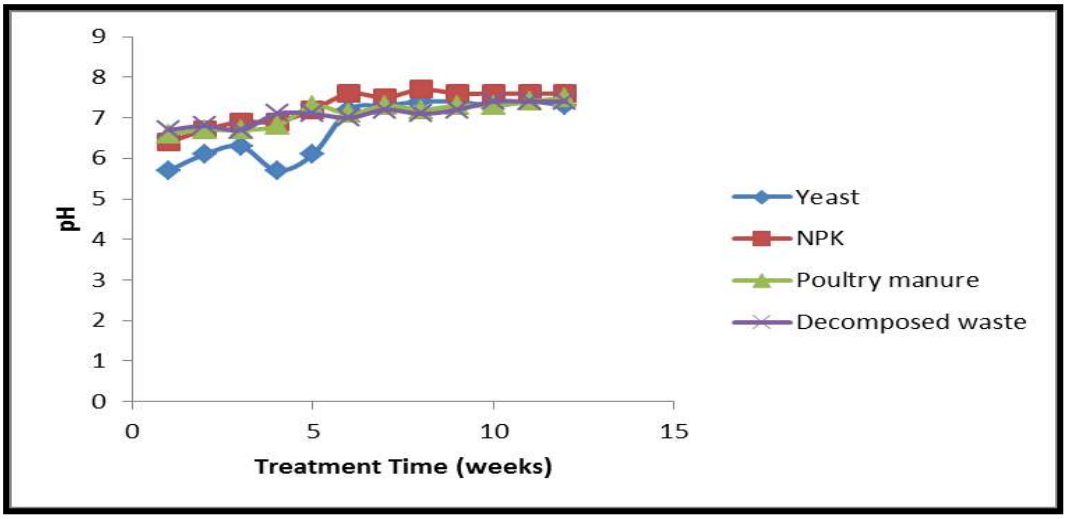

Figure 4: Variation of pH with treatment time

Results of electrical conductivity and total dissolved solids as presented in Figures 5 and 6 reveals the presence of more mobile dissolved solute resulting from the breakdown of crude petroleum hydrocarbon by the substrate utilizing microorganism at the early stages of the remediation process.

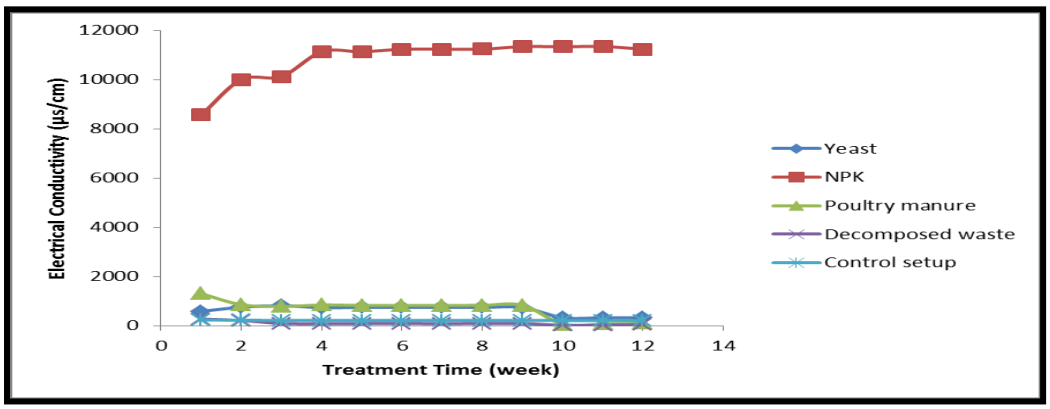

Figure 5: Variation of electrical conductivity with treatment time

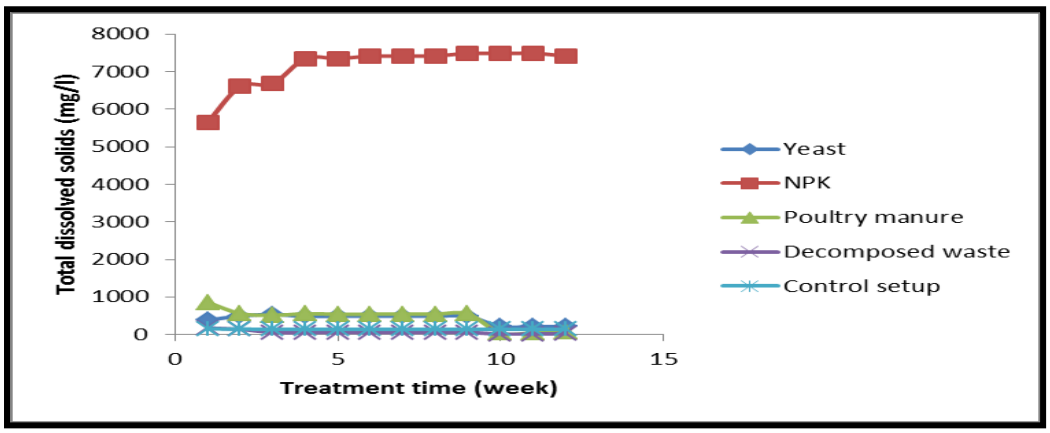

Figure 6: Variation of total dissolved solids with treatment time

It is suspected that the breakdown of petroleum hydrocarbon results in the formation of by products that are ionic in nature and capable of conducting electricity when they undergo catalytic dissociation. This reaction will consequently increase the level of conductivity of the entire process of remediation while also increasing the amount of total dissolved solids. This trend was observed with all the substrate throughout the period of experimentation.

The amount of petroleum hydrocarbon removed during the series of batch investigation including the efficiency of remediation was determined using the mass balance equation adopted from Raghuvanshi et al, (2004) and results obtained is presented in Figures 7 and 8 respectively.

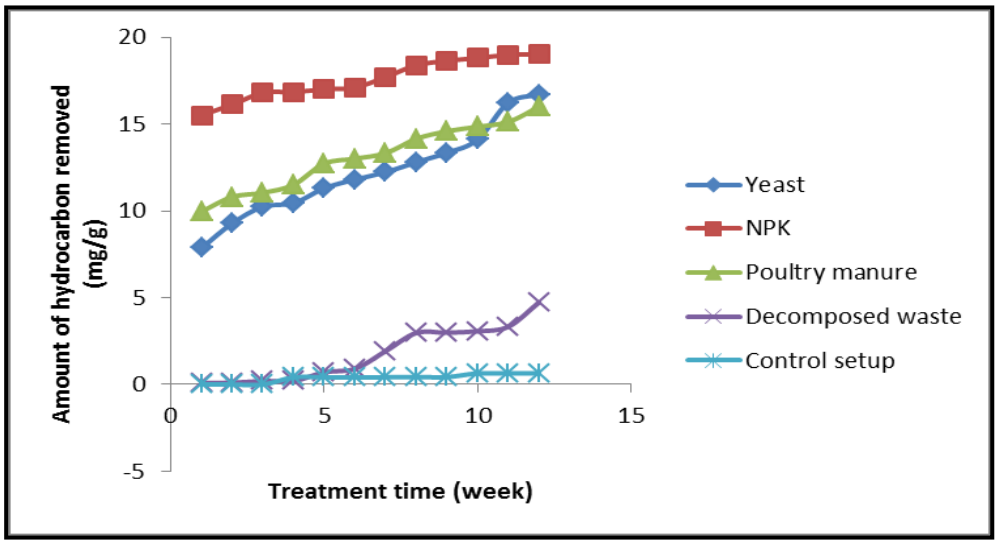

Figure 7: Amount of petroleum hydrocarbon removed with time

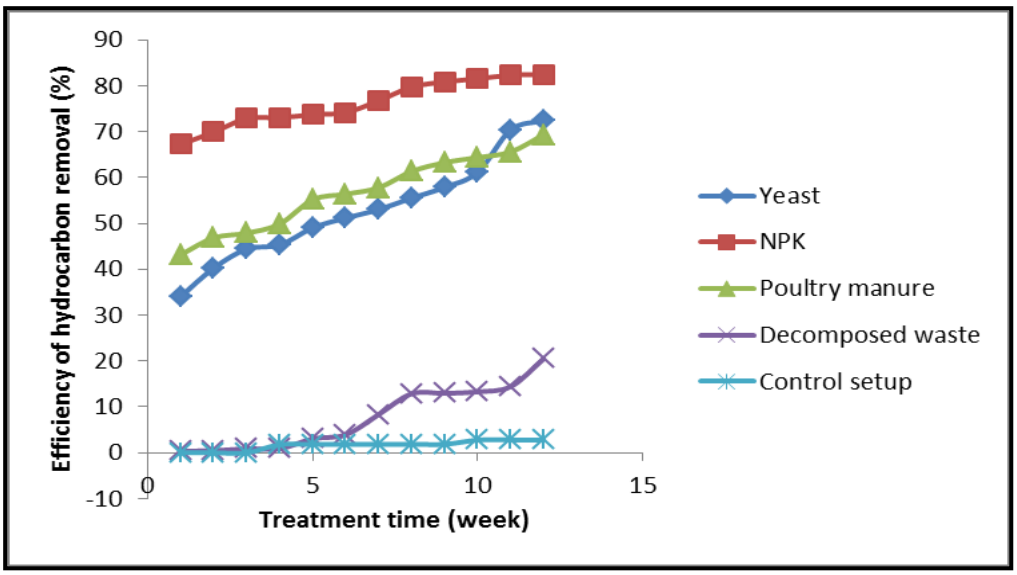

Figure 8: Efficiency of petroleum hydrocarbon removal with time

It can be deduced from the result of Figure 8 that, the efficiency of remediation increases with treatment time. Increase in the efficiency of remediation with treatment time can be attributed to the increase in the population of the microorganism resulting from the gradual decrease in the available nutrients (nitrogen and carbon respectively).

4.0 Conclusion

From the results and analysis, it was observed that the use of nutrient agar and microorganisms from selected substrates on crude petroleum hydrocarbon polluted soils facilitate the rate of hydrocarbon degradation, a process popularly referred to as bioremediation.

The extent of remediation was judged based on change in the concentration of some selected parameters with time. The selected parameters include; pH, electrical conductivity, Tds, organic carbon, total nitrogen, and the total hydrocarbon content. Based on the study, the following conclusion were drawn:

a. It was observed that for the entire period of experimentation (12 weeks), there occur a gradual decrease in the total nitrogen content (TNC), total organic carbon (TOC) and total hydrocarbon content (THC).

b. As the microorganism utilizes the available nitrogen and organic carbon present in the soil and increase in population, they again react with and break down the agent that causes the pollution which is the petroleum hydrocarbon

c. It was observed that the experimental data are approximately normally distributed and dead cell microorganism (Yeast) was seen to be the best substrate for the bioremediation of hydrocarbon polluted soil followed by poultry manure, NPK and finally decomposed waste.

The study has provided additional information on the performance of yeast, inorganic substrate such as NPK, organic substrate such as poultry manure and decomposed waste in the bioremediation of hydrocarbon polluted soil.

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