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Original Research

Stimulated biodegradation of waste lubicating oil in soil, using water hyacinth and goat droppings

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ABSTRACT

Background: Soil contamination with used engine oil is becoming a major environmental problem due to uncontrollable disposal, particularly in developing countries. *Eichhornia Crassipes* (water hyacinth) has been recognized as the world's most harmful weed. The aim of this research is therefore to determine the potentials of water hyacinth and goat droppings in enhancing biodegradation of used engine oil in soil. **Materials and methods:** Water hyacinth plants were chopped, sun dried and ground into powder. It was autoclaved at 121^{0}C for 15 minutes. 200g of Soil was placed in vessels labelled A,B and C, 2.5% of used lubricating motor oil was added, pulverised, and left undisturbed. After 2 days 10% of each organic waste (ground water hyacinth and goat droppings) was added to each vessel of oil polluted soil, labeled A and B. Vessel C with only soil and used lubricating oil served as control. Periodic sampling was carried out at 15 days interval for 75 days. Soil samples were collected from different areas of the microcosm for isolation and enumeration of bacteria and determination of total petroleum hydrocarbon. **Results:** Microbial growth was highest in contaminated soil amended with water hyacinth with total heterotrophic bacteria count ranging from 20×107 to 68×107 cfu/g and total hydrocarbon utilizing bacteria count ranging from 10×106 to 40×106cfu/g. This was closely followed by soil amended with goat droppings with total heterotrophic bacteria count ranging from 18.8×107 to 60×107cfu/g and hydrocarbon utilizing bacteria count ranging from 8.5×106 to 38×106cfu/g. Unamended soil had the lowest microbial count with total heterotrophic bacteria count ranging from 4.0×107 to 7.2107cfu/g and 7.5×106 to 20×106cfu/g for hydrocarbon utilizing bacteria count. 91% loss in hydrocarbon was observed in the water hyacinth amended soil. **Conclusion:** The biostimulative effect was observed with the increase in microbial population count in contaminated soil amended with water hyacinth. This study confirms that water hyacinth and goat droppings have the potential to stimulate bioremediation of hydrocarbon polluted soil.

KEY WORDS: Biodegradation; Water hyacinth; Soil

INTRODUCTION

The pollution of soil and subsurface environment by petroleum products is of major concern in the industrial world [1]. Large amount of hydrocarbon contaminants are released into the environment as a result of human activities [2]. Petroleum spills may persist in the soil as a source of hazardous hydrocarbon for a long time (months or years) because of low solubility and the moderate of low volatility of these compounds [3].

Petroleum hydrocarbon can affect soil ecosystems sufficiently to result in significant looses in soil quality [4,5]. Their negative impact results from their toxicity of biological processes catalysed by soil micro organism. Field studies of contaminated soil microorganisms have demonstrated the elevated loading of these contaminants can result in diminished microbial biomass, reduced valuable bacterial population densities, inhibition of organic matter mineralization and decreased leaf litter decomposition [6].

Engine oil is a complex mixture of hydrocarbon and other organic compounds including some organo-metalic constituents [7], that is used to lubricate the parts of automobile engine, in order to avoid excessive wearing out [8]. As it is inevitable for the efficient functioning of automobile engines, soil contamination with used engine oil is becoming one of the major environmental problems due to uncontrollable disposal particularly in developing countries [9]. Waste motor engine oil contains metals and heavy polycyclic aromatic hydrocarbon (PAH) and they could contribute to chronic hazards including mutagenicity and carcinogenicity [10]. Some of the metals in used motor oil can dissolve in water and move through the soil easily, and may move through the soil easily, and may be found in surface water and ground water. Thus, metals in used oil can build up in plants, animals and soil, sediments and non flowing surface water. Heavy metals and chemicals in used motor oil are absorbed and distributed into various tissues in human, plants and animals by their movement in the environment, which can result in serious health problem, such as anaemia, tremor, and consequently resulting in death [11].

Many remediation technologies have been developed to treat soil contaminated by petroleum pollutants. Mechanical and chemical methods have limited effectiveness and can be expensive. Several researches have studied the use of micro organism to decompose petroleum products and have shown this to be promising technological alternative [12,13].

Bioremediation uses biodegradation to achieve its goal as it is defined as any process that uses micro organisms or their enzymes to return the environment altered by contaminants to its original condition [14].

A wide range hydrocarbon utilizers (HCUs) found to be useful in soil include the following species: *Pseudomonas*, *Rhodococcos*, *Mycobacterium*, *Strephococcus* [15]. Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent, but they take longer periods when compared to their bacterial counter parts [16].

Water hyacinth (*Eichhornia crassipes*) is a free floating plant from the family Pontederiacea, that grows in fresh water but may be rooted in the mud from where it derives all it nutrient directly. Water hyacinth has been recognized as the world's most harmful weed due to its negative impact on people's livelihood and waterways. Efforts to control the weed through physical, chemical, and biological methods have met with little success. However, there is a continued theme from researchers that there is significant benefit to be obtained from seeing water hyacinth as a resource rather than a rogue plant. Water hyacinth was reported by [17] as an organic fertilizer as a source of limiting nutrient for the bioremediation of hydrocarbon polluted swamp in the Niger Delta, Nigeria.

Biostimulation using inorganic fertilizer has been extensively employed worldwide in reclaiming oil polluted soil [18]. The use of inorganic fertilizer has been extensively carried out. However, the use of inorganic fertilizer is still challenged by the large cost of bioremediation and the likely chance of eutrophication/algae bloom especially in the aquatic environments. The use of organic source of nutrient such as chicken droppings, periwinkle shells, cow dung for the bioremediation of crude oil polluted environment other than mangrove swamp has been reported in Nigeria [19,20]. The aim of this research is therefore to determine the potentials of water hyacinth and goat droppings in enhancing biodegradation of used engine oil in soil.

MATERIALS AND METHODS

Sample collection and preparation

The unpolluted soil was collected randomly from different spots to form a composite with a soil hand dug auger at a depth of 5-15 cm at the botanical garden at Yaba College of Technology, Lagos, Nigeria. The soil samples were transported to the laboratory, air dried and sieved through a 2mm mesh sieve. This site was chosen because it is free from any form of hydrocarbon contamination and industrial activities. All soil used for this research work was collected from this location. Spent lubricating motor oil used for this research work was collected from a drained motor engine from automobile repair service station at Ladipo automobile market, Lagos, Nigeria. Water hyacinth Plants were collected from Makoko, Lagos, Nigeria. It was chopped into pieces, sun dried for 6 days, ground into powder form and autoclaved at 121^0c for 15 minutes. Goat dropping used was collected from an animal farm at Surulere, Lagos, Nigeria. It was sundried for 5 days after which it was autoclaved at 121^0c for 15 minutes. The physiochemical properties were determined for all the samples. The study was carried out in 2015.

Microcosm set up description

Soil (200g sieved through a 2mm mesh size) was placed in vessels labeled A to C, 2.5% of used lubricating motor oil was added, pulverised, and left undisturbed for 2 days. The aim of this contamination was to simulate condition of a major spill [21]. The polluted soils with lubricating oil were amended with dried water hyacinth and goat droppings. After two days 10% of each organic waste (ground dried water hyacinth and goat droppings) were individually added to each vessel of oil polluted soil labeled A and B respectively and thoroughly mixed. The moisture content was adjusted to 60% water holding capacity and incubated at room temperature (28 ± 20^oc). Vessel C contain unpolluted soil and used lubricating oil which served as control. The control was never amended with nutrients. The content of each vessel was tilled twice a week for aeration, and the moisture content was maintained at 60% water holding capacity by adding sterile distilled water. The experiment was set up in triplicate. Sampling for laboratory studies started immediately after the soils were amended with nutrients.

Biodegradation studies

Periodic sampling of each vessel was carried out at 15 days interval for 75 days that is at Day 0, 15, 30, 45, 60 and 75. Composite samples were obtained by mixing 5g of the soil collected from different areas of the microcosm, for isolation and enumeration of bacteria and determination of total hydrocarbon content.

Determination of Physiochemical parameter of Soil, Water Hyacinth and Goat droppings

Phosphate was determined using the HACH DR 2000 direct reading spectrophotometer, method 8048 [22]. Nitrate was determined using HACH DR 2000 direct reading spectrophotometer method 8039 [22]. Moisture content was determined using Walkey and Black Rapid Titration method [22]. PH was determined using testr-2 PH meter [22]. Organic carbon was determined using the wet oxidation method described by [23].

Total Hydrocarbon Content Determination.

The Total Hydrocarbon Content was estimated by gravimetric method in which aliquot portion of the soil sample was extracted using n-hexane twice, dried anhydrous sodium sulphate crystals. The dried extract was collected in clean, dried and weighed beaker and evaporated to almost dryness and the drying process completed in a desicator. The THC is the mass difference between the masses of the

empty beaker and the beaker with the THC [22].

Microbiological analysis

Enumeration of heterotrophic bacteria count.

The replicates samples from each oil polluted soil were withdrawn every 15 days for enumeration of heterotrophic bacteria count. Serially diluted samples (0.1ml) were plated on nutrient agar medium. The plates were incubated at 30^0c for 24 hours before the colonies were counted.

Enumeration of hydrocarbon utilizing bacteria.

Hydrocarbon utilizing bacteria in the soil samples were enumerated using modified mineral salt medium [24]. The composition includes 1.8g k_2HP0_4,4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 0.001$FeSO_4.7H_20$, 0.1g of Nacl, 20g agar in 1000ml of distilled water PH 7.4. The transfer method was used, a filter paper saturated with sterile oil was aseptically placed on the inside of a petri dish and culture plate were incubated at (28 ± 2^0c) for 7days. Colonies of different hydrocarbon utilizing bacteria were randomly picked and pure culture bacteria isolates were identified using Bergey's manual of determinative microbiology.

Characterization and identification of bacteria

Bacteria are known to exhibit distinctive biochemical reaction or characteristics when supplied with certain biochemical substances. Their ability to exhibit these biochemical characteristics is inherent in bacteria ability to elaborate necessary enzymes. Therefore for bacteria to be fully identified and characterized Biochemical tests are very essential. Biochemical tests used to determine bacteria in this study include: Glucose, lactose, Hydrogen sulphide gas production, catalase, coagulase, motility, indole, urease, citrate and oxidase.

Calculation of percentage loss in THC

Percentage loss in THC was calculated using the formula below:

$$\% \text{ Loss in THC} = \frac{\text{Concentrationat at point - Conc. at time zero X 100}}{\text{Concentration at time zero}}$$

Statistical Analysis

Statistical analyses were carried out using Statistical Package For Social Sciences (SPSS, Version 17.0). Statistical analysis of data was carried out using analysis of variance (ANOVA), Least Significant Difference (LSD), and Correlation Coefficient Methods. These were used to determine the relationship between the variables.

RESULTS

Physiochemical studies of soil and organic waste

The results of the physiochemical properties of soil and organic waste (water hyacinth and goat dropping) are shown in Table I. The soil had a pH of 8.79 which is alkaline, the pH of each of the organic wastes were 8.84 and 10.90 for water hyacinth and goat droppings respectively.

Biochemical tests of bacterial isolates

The result of the biochemical test is presented in Table II. The isolates were negative for urease and positive for Hydrogen sulphide gas production and glucose except isolate 4. Isolates exhibited negative gram reaction and showed negative for lactose (Table II).

Table I. Physiochemical properties of soil and organic wastes

Parameters	Soil (mean values)	Water hyacinth (mean values)	Goat droppings (mean values)
PH	8.79	8.84	10.90
Nitrogen (mg/kg)	13.40	54.0	17.90
Phosphorus (mg/kg)	7.69	26.16	22.40
Organic carbon (%)	10.00	6.54	29.90
Moisture (%)	6.90	12.96	8.80

Table II. Biochemical identification of bacterial isolates

Isolate number	Gram Reaction	Glucose	H_2S Gas production	Lactose	Motility	Indole	Urease	Citrate	Oxidase
Isolate 1	-	+	+	-	+	-	-	+	-
Isolate 2		+	+	-	+	-	-	-	-
Isolate 3	-	+	+	-	-	-	-	-	+
Isolate 4	-	-	-	-	-	-	-	-	+
Isolate 5	-	+	+	-	+	+	-	+	+

Table III. Cultural characteristics and identities of isolates associated with biodegradation of waste lubricating oil in soil.

Isolate number	Culture characteristics	Bacterial identity
Isolate 1	It has orange pigmentation, it is raised and opaque and gram negative.	*Bacillus Spp*
Isolate 2	It has no pigmentation, its white, dry, raised and opaque and gram positive.	*Rhodococcus Spp*
Isolate 3	It has no pigmentation its white, flat, opaque and gram negative.	*Flavobacterium Spp*
Isolate 4	It has no pigmentation it is white flat gram negative.	*Micrococcus Spp*
Isolate 5	It has green pigmentation its flat transluscent and gram negative.	*Pseudomonas Spp*

Cultural characteristics and identities of isolates associated with biodegradation of waste lubricating oil in soil.

The hydrocarbon utilizing bacterial isolates obtained from this study included *Bacillus Spp*, *Rhodococcus Spp*, *Flavobacterium Spp*, *Micrococcus Spp*, and *Pseudomonas Spp* (Table III). The cultural characteristics of the isolates are presented in Table III.

Microbial Counts

Total heterotrophic bacteria count

Figure I shows the total heterotrophic bacteria count monitored on Day 0, 15, 30, 45, 60 and Day 75 for the different soil types investigated (amended and unamended soils). Total heterotrophic bacteria in polluted soil amended with water hyacinth ranged between 20.0x 10^7 and 68.0 x 10^7 Cfu/g and that of the polluted soil amendment with goat droppings ranged from 18.8 x 10^7 to 60.9 x 10^7 Cfu/g. The total heterotrophic bacteria count in the unamended polluted soil ranged between 4.5x10^7-12.0x 10^7 Cfu/g as shown in Fig I.

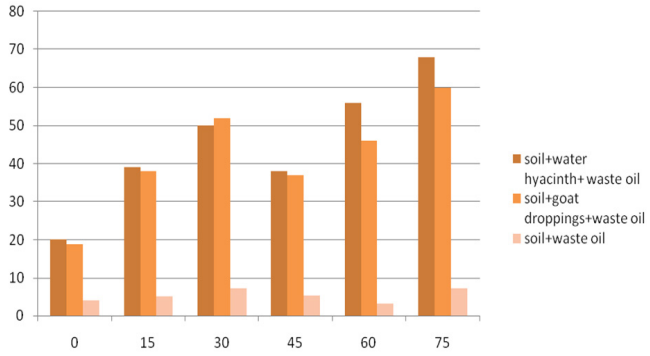

Figure I. Total heterotrophic bacteria count for a period of 75 days

Total hydrocarbons utilizing bacteria count.

Figure II shows the total hydrocarbon utilizing bacteria count monitored on Day 0, 15, 30, 45, 60 and Day 75 for the different polluted soil types investigated (amended and unamended soil). The counts of hydrocarbon utilizing bacteria in the polluted soil amended with water hyacinth ranged from 10.0x10^6 to 40.0 x 10^6 Cfu/g while that of the polluted soil amended with goat droppings ranged from 8.5 x10^6 to 38.0 x10^6 Cfu/g. Hydrocarbon utilizing bacteria count in unamended polluted soil was between 4.5x10^6 and 12.0 x10^6 Cfu/g.

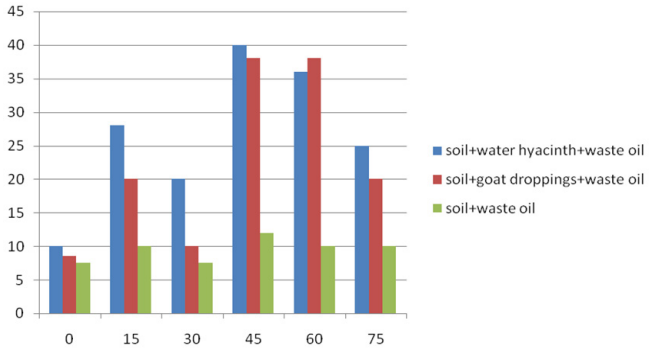

Figure II. Total hydrocarbon utilizing bacteria count for a period of 75 days

Total hydrocarbon content in amended and unamended polluted soils

The Total Hydrocarbon Content (THC) in the water hyacinth amended polluted soil, decreased at day 0 from 18878.79 mg/kg to 1735.30 mg/kg after 75 days (Fig III). In the polluted soil amended with goat droppings, the decrease observed from day 0 to day 75 was 18878.79 mg/kg to 1956.50 mg/kg. The changes in the total hydrocarbon content observed in unamended soil was from 18878.79 mg/kg (day 0) to 5561.17 mg/kg (day 75) (Fig III).

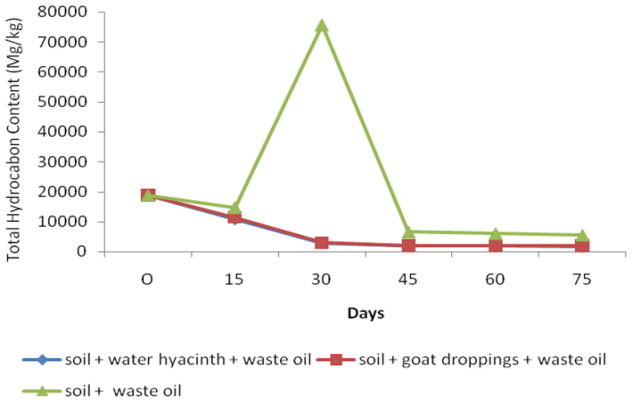

Figure III. Total heterotrophic bacteria count for a period of 75 days

Percentage loss in total hydrocarbon content in amended and unamended polluted soils

The percentage loss in Total Hydrocarbon Content (THC) on the zero day was 0% for the three conditions. However, for Water hyacinth amended experimental set-up, percentage Total Hydrocarbon Content (THC) lost was 41.99% to 90.80% between the 15th day to 75th day respectively (Fig. IV). In the goat droppings amended experimental set-up, 39.19% to 89.64% of THC were lost between the 15th day and 75th day of the study respectively (Fig. IV).

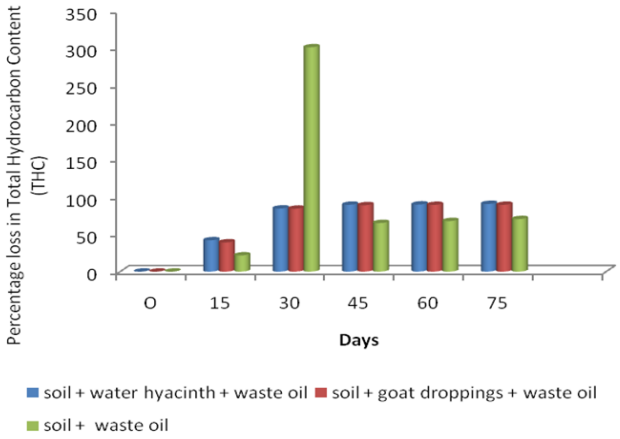

Figure IV. Total heterotrophic bacteria count for a period of 75 days

DISCUSSION

The physiochemical properties of soil and organic waste (water hyacinth and goat dropping) indicated that the soil had a pH of 8.79 which is alkaline, the pH of each of the organic wastes were 8.84 and 10.90 for water hyacinth and goat droppings respectively. In a similar study [25], recorded a pH of 8.75 for water hyacinth. The nitrogen and phosphorus content were 13.40mg/kg and 7.69mg/kg respectively for the soil samples. These were lower than those of the organic wastes. Nitrogen and phosphorus content were 54.0mg/kg and 26.2mg/kg respectively for water hyacinth. It was observed that the water hyacinth was rich in nitrogen and phosphorus. Nitrogen and phosphorus content for the goat droppings were 17.90mg/kg and 22.40mg/kg respectively. Moisture content was higher in water hyacinth (12.96%) and goat droppings (8.80%), than the soil (6.90%). Goat droppings were rich in organic carbon (29.90%). Statistical analyses showed that there was statistical significance at the $p<0.05$ for the three samples. Water hyacinth possesses natural ability to absorb vast amounts of nutrients into its tissues causing formation of nutrient sinks, which essentially removes these nutrients from the ecosystem [26]. This phenomenon is reflected in the observation that nitrogen and phosphorus contents were relatively higher in water hyacinth compared to goat droppings and soil.

The result of the biochemical test shows that the isolates were negative for urease and positive for Hydrogen sulphide gas production and glucose except isolate 4. Isolates exhibited negative gram reaction and showed negative for lactose.

The hydrocarbon utilizing bacterial isolates obtained from this study included *Bacillus Spp*, *Rhodococcus Spp*, *Flavobacterium Spp*, *Micrococcus Spp*, and *Pseudomonas Spp*. The cultural characteristics of the isolates indicated that these species have been implicated as hydrocarbon utilizers as reported by [14]. *Pseudomonas* species, *Bacillus* species, *Citrobacter* species, *Micrococcus* species, *Vibrio* species, *Flavobacterium* species and *Corynebacterium* were obtained as hydrocarbon utilizing bacterial isolates in a study by [17].

The total heterotrophic bacteria count monitored on Day 0, 15, 30, 45, 60 and Day 75 for the different soil types investigated (amended and unamended soils). Total heterotrophic bacteria in polluted soil amended with water hyacinth ranged between 20.0x 10^7 and 68.0 x 10^7 Cfu/g and that of the polluted soil amendment with goat droppings ranged from 18.8 x 10^7 to 60.9 x 10^7 Cfu/g. The total heterotrophic bacteria count in the unamended polluted soil ranged between 4.5x10^7-12.0x 10^7 Cfu/g. The heterotrophic bacteria count in the unamended soil was the lowest compared to the amended soil. The soil amended with water hyacinth had the highest heterotrophic bacteria count. Day 75 recorded the highest count of total heterotrophic bacteria for both amended polluted soils. The total heterotrophic bacteria counts showed that there was general increase in the entire vessels even in the control, unamended and the total hydrocarbon utilizing bacteria count increased in similar manner although the control vessel had the least count, similar trend was reported by [27]. The reason for higher counts of bacteria in amended soil may be due to high amount of nitrogen and phosphorus in water hyacinth and goat droppings. This nutrient if limiting can limit biodegradation in terrestrial environment, this was also reported by [28].

The Total Hydrocarbon Content (THC) in the water hyacinth amended polluted soil, decreased at day 0 from 18878.79 mg/kg to 1735.30 mg/kg after 75 days. In the polluted soil amended with goat droppings, the decrease observed from day 0 to day 75 was 18878.79 mg/kg to 1956.50 mg/kg. The changes in the total hydrocarbon content observed in unamended soil was from 18878.79 mg/kg (day 0) to 5561.17 mg/kg (day 75). Total reduction in petroleum hydrocarbon may not be due to biodegradation process alone induced by the addition of nutrient but also by volatilization, adsorption to organic compounds and abiotic factor are equally implicated in the process. In a biostimulation laboratory model by [29] using NPK fertilizer the total hydrocarbon at baseline (1000 mg kg-1) reduced to 600 mg kg-1, 586 mg kg-1 560 mg kg-1 and 387.21 mg kg-1 for zero hour, 14th day, 28th day, 42nd day and 56th day respectively [30] also reported that the Total Hydrocarbon Content (THC) lost in autoclaved control was not greater than 120 mg kg-1. This little amount of THC lost can be accounted for by natural attenuation process

such as photo-oxidation, volatilization and evaporation. It is not surprising that at zero hour in all the experimental groups, there was no loss of either TPH or THC. This is because at the zero hour, microbial metabolism has not actually begun [17].

The hydrocarbon utilizing bacteria count in the unamended soil was the lowest compared to the amended soil. The soil amended with water hyacinth had the highest hydrocarbon utilizing bacteria. Day 45 recorded the highest count of total hydrocarbon utilizing bacteria for both amended and unamended polluted soils. The hydrocarbon utilizing bacteria was observed to decrease after the 45th day. The addition of water hyacinth and goat droppings rich in nutrients is responsible for the increase in hydrocarbon utilizing bacteria count recorded in this study. Water hyacinth is known for its rapid growth, environmental and social problems, its effective use in biodegradation of polluted soil will go a long way in managing the plant. The hydrocarbon utilizing and heterotrophic bacteria responded positively to the biodegradation treatment with higher population occurring in the amended set up as time elapsed. The bacterial species exhibited ability to either degrade or utilize the different petroleum hydrocarbon components as sole carbon sources. Similarly, [17] in a bioremediation study observed that the hydrocarbon utilizing bacterial species responded to the nutrient amendment with water hyacinth recipe. The total culturable hydrocarbon utilizing bacterial count in water hyacinth experiment increased at zero hour from $2.5x10^4$ Cfu/g to $3.7x10^7$ Cfu/g during the period of study that lasted for seventy (70) days. In addition, in another study in a contaminated sandy loamy soil in Port Harcourt, [29] documented the use of poultry manure to increase the hydrocarbon utilizing bacteria count significantly.

CONCLUSION

The result of this research have shown that as much as water hyacinth defines itself a nuisance, and goat dropping being under estimated and considered waste they can both be used as source of limiting nutrient in remediating hydrocarbon contaminated environment. Biostimulation using water hyacinth is an effective approach of bioremediation which is a simple, eco-friendly process that depends solely on natural ability of microorganism.

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