International Research Journal of Natural Sciences 13 (1),16-26, 2025
Print ISSN: ISSN 2053-4108(Print)
Online ISSN: ISSN 2053-4116(Online)
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Efficacy of Water Hyacinth (*Eichhornia Crassipes*) Compost and Spent Mushroom Substrate on Bioremediation of Crude Oil Polluted Soil

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doi: https://doi.org/10.37745/irjns.13/vol13n11626 Published March 29, 2025

Citation: Ngbara-ue K.A., Stanley H.O., Onwukwe C.D., Vincent-Akpu I.F., Abu G.O. and Akwukwaegbu R.N. (2025) Efficacy of Water Hyacinth (Eichhornia Crassipes) Compost and Spent Mushroom Substrate on Bioremediation of Crude Oil Polluted Soil, *International Research Journal of Natural Sciences*, 13 (1), 16-26

Abstract: *Oil exploration and exploitation activities in the Niger Delta region has brought about contamination of the environment through accidental discharges and sabotage further introducing toxic hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs), into the ecosystem. The aim of this study is to achieve measurable restoration of a crude oil contaminated soil to its natural state through bioremediation technology using water hyacinth (Eichhornia crassipes) compost and spent mushroom substrate. The standard spread plate technique was used to study the growth dynamics of microorganisms. Bacillus sp (19.40%), Proteus sp (4.48%), Pseudomonas sp (13.43%), Serratia sp (4.48%), Micrococcus sp. (13.43%), Arthrobacter sp. (21.00%), and Staphylococcus sp. (23.88%) were the bacterial isolates identified whereas Aspergillus sp. (55.56%), Saccharomyces sp. (13.33%), Penicillium sp. (4.44%), Fusarium sp. (17.78%), and Rhodotorula sp. (8.88%), were the fungal isolates identified in the study. TPH reduced by 91.69% in Sterile soil +Crude oil + water hyacinth compost (SCWH), 81.94% in Sterile soil + Crude oil + Spent mushroom substrate (SCSM). All heavy metals studied had*

decreased concentration in the treatments within the period. This research looked into waste utilization for a cleaner environment as reckless dumping of these plant wastes constitute environmental nuisance. Cultivation of crops like groundnut, hamburger bean and mushroom farming should be encouraged as their wastes serve as potential candidate for remediation of polluted environment.
Keywords: exploration, Hydrocarbon, Polycyclic Aromatic Hydrocarbon and spent mushroom substrate

INTRODUCTION

Water hyacinth, or *Eichhornia crassipes*, is an aquatic plant that has drawn a lot of attention due to its capacity to quickly absorb toxins from aquatic habitats. The best management approach is to find some use for them, since attempts to regulate it have not been entirely successful. The main potential uses of water hyacinth are the production of biogas and bioethanol, biosorbent for the removal of harmful metals (Malik, 2007), and animal fodder/fish feed (Aboud et al., 2005). Additionally, water hyacinth can be utilized to recover some harmful and non-biodegradable elements, such as heavy metals, after pollutants have been removed from waste water (Isarankura-Na-Ayudhya. et al., 2007). Water hyacinth's qualities, including its faster growth rate, great effectiveness in absorbing pollutants, cheap operating costs, and renewability, make it a viable solution for treating wastewater. According to Malik (2007), water hyacinth poses significant problems for irrigation, producing electricity, and transport. Therefore, water hyacinth removal and the application of phytoremediation techniques are necessary to prevent these issues. Additionally, he discovered that certain aquatic plants, such as water hyacinth, can be utilized to produce biofuels. Dried water hyacinth can become utilized to make briquettes, which are utilized for simultaneous combustion in coal power plants. The primary cause of the massive amounts of wastewater that are distributed into the surroundings is people growth, development, and industrial development, which primarily consists of organic materials and heavy metals. Therefore, a dependable technological device is required to treat wastewater before it is published into the water bodies. Although wastewater treatment technologies are often expensive, they are not always environmentally friendly, so researchers around the world have been paying more attention to environmentally friendly technologies. According to Rezania et al. (2015), the use of phytoremediation strategies to treat various wastewater types has been documented by numerous studies. A variety of contaminants, including biochemical oxygen demand, heavy metals, total suspended solids, chemical oxygen demand, dissolved solids, nitrogen, and phosphorus, have been removed using water hyacinth and water lettuce. Rezania and associates (2015) only a small number of review publications about water hyacinth-based wastewater treatment processes have been released recently (Rezania et al., 2015). This study primarily focuses on the most recent

research conducted over the last five years on the uptake and removal of organic, inorganic, and heavy metals from storm water using water hyacinth, making it an appropriate, affordable, efficient, and environmentally friendly wastewater treatment technique. This review's primary goal is to assess the efficacy of water hyacinth to other aquatic plants in terms of removing contaminants from wastewater and to offer guidance for the creation of new, cutting-edge phytoremediation methods.

Control of water hyacinth

There are a number of widely used control strategies to stop water hyacinth from spreading or becoming extinct. Physical, chemical, biological, and run-off control are the four primary processes. Each has advantages and disadvantages. Because of its unknown long-term consequences on the environment and the communities it comes into touch with, chemical management is the least preferred method. Although physical control—which includes the use of dredgers, automated mowing machines, and manual extraction techniques—is frequently employed, it is expensive and ineffective for big infestations. It is typically thought of as a temporary fix and is not appropriate for major infestations. The most popular long-term control strategy is biological control, which is also quite simple to implement and may be the only sustainable and cost-effective option available (Henderson 2001).

***Roles of Water Hyacinth in bioremediation*:** The propensity of water hyacinth to absorb mineral compounds is well documented. According to earlier research, it can absorb heavy metals like cadmium and zinc (Henderson 2001) and remove up to 70% of the chromium in wastewater (Keith et al., 2006). The exotic water hyacinth plant can reach a height of three feet and features green, sharp-edged leaves that are round to oval in shape and attached to a spongy rhizome. In many tropical and subtropical climates, water hyacinth thrives, albeit some can become problematic. Thankfully, this also implies that the area with those plants contributes to the pollution removal of its rivers and lakes. Prior research has demonstrated that metal uptake time and detention area are critical factors in phytoremediation, especially rhizofiltration. Rhizofiltration is the process by which heavy metals are filtered and absorbed by plant roots over a predetermined amount of time. Because there is water available to store pollutants, a plant's response to hydraulic retention time (HRT) is also taken into account. As a result, the current treatment approach was chosen from an economic perspective, and it may be used to implement in situ plants that grow on the surface of ceramic wastewater (Asif & Zhang, 2021).

Spent mushroom substrate

As visible spore-bearing structures known as sporocarps, mushrooms play a crucial role in the sexual reproductive stage of many fungi's life cycles (Hays & Watson, 2019). Because they are high in proteins, dietary fiber, vitamins, minerals, and other nutrients and low in toxins and anti

nutrients, many mushrooms are regarded as edible. The species determines the particular makeup of mushrooms. According to Bellettini et al. (2019), mushrooms can contain up to 30% (w/w) crude protein, while some species can have up to 28, 8, and 95% (w/w) of crude fiber, fat, and carbohydrates, respectively. The term "medicinal mushrooms" is also used to refer to edible mushrooms, which are climate-smart, protein-rich food sources that can partially replace meat and whose production has a significant impact on the climate due to their high content of various health-promoting ingredients, such as β-glucans, peptides, proteins, and phenolic compounds. Spent mushroom substrate (SMS) is the residual biomass generated after harvesting the fruit bodies of edible/medicinal fungi. Spent mushroom substrate (SMS) is the soil-like material left over after a crop of mushrooms; it is high in organic matter, making it desirable for use as a soil amendment or soil conditioner.

Spent mushroom as substrate of bioremediation

Bioremediation is a promising technique for the amelioration of soils contaminated with TPHs. However, for the heavier hydrocarbons, the efficiency of this technique is limited due to their low bioavailability, which makes bacterial degradation difficult (Maletic. et al., 2011), since these organisms have to absorb the contaminant to degrade it intracellular. To solve this problem, a new procedure known as mycoremediation has been proposed. In this approach, fungi are applied to soil for enhancing contaminants removal (Chukwunonso. et al., 2020). SMS wastes are based on lignocellulosic materials and contain viable mycelium and specific microbiota able to colonize polluted soils and degrade a wide variety of soil contaminants. The re-use of SMSs in environmental remediation is a promising way to covert this agricultural waste into a sustainable bioresource promoting the green development of the global mushroom industry. The composted SMS is an effective adsorbent to minimize the availability of metals in soil and enhanced the phytoremediation of mining soil reduced the leaching of pesticides from soil and minimized the impact of herbicides in soil microbial community. The ability of SMS to remove a wide variety of organic contaminants from soils was reported in different publications (Chukwunonso. et al., 2020).

Composition of spent mushroom substrate

The rest product of the industry producing button mushrooms, *Agaricus bisporus*, is called Spent Mushroom Substrate (SMS), SMS consists of a mixture of two substrates been used: one, compost for the nutrition of mushroom mycelium, and two, ‘casing soil’ that allows the development and growth of fruit bodies. The main ingredients for compost are straw, straw (rich horse manure, chicken manure and gypsum. The main ingredients for casing soil are peat and sugar beet lime. Spent Mushroom Substrate (SMS) contains about 14% protein and abundance of vitamins and micro elements such as Fe, Ca, Zn and Mg and have been used to enhance yield of crops (Chukwunonso. et al., 2020).

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MATERIALS AND METHODS

Collection and Preparation of Raw Materials

Plate 1: Spent mushroom Substrate

Plate 2. Water hyacinth compost

Plate 3: pristine soil

Spent mushroom substrate was gotten from a demonstration farm in Faculty of Agriculture, University of Port Harcourt. The big lumps as it was were broken down into chaff, a form fit for application into crude oil contaminated soil for remediation purposes.
The water hyacinth compost used in this research was collected from a larger quantity gathered by a research team domiciled in the green house, University of Port Harcourt.
The soil sample used in this research was collected from a heap located between Ofrima and Animal & Environmental Biology (AEB) buildings, faculty of Science, Abuja Park, University of Port Harcourt, using a trowel. Sample was collected at 15 cm depth, mixing the top a bottom samples to obtain a composite sample. The soil was exposed at room temperature to reduce its high moisture content through evaporation, sieved to remove stones and other rocky particles in order to obtain a smooth soil. The reason for this was to allow for uniform or fine distribution of hydrocarbon contaminant.

Microbiological Analysis

Microbiological analysis deployed in this study includes both quantitative and qualitative analysis. The quantitative aspect deals with the enumeration of the population of microorganisms whereas the qualitative aspect deals the biodiversity of the samples (treatments). The microbiological analysis done detailed in the following headings;

(1) Inoculation: four microbiological parameters were considered in this study. Step-by-step breakdown of the procedures for each are explained below

Total Heterotrophic Bacteria Count (THBC)

The method described by APHA 9215C was adopted in this study. The medium nutrient agar (NA) was used for this analysis. The medium was prepared according manufacturer's direction by weighing and dissolving 28g/l, sterilize by autoclaving at 121^0C for 15 minutes, allowed to cool to about 45^0C, poured into sterile petri dishes and allowed to solidify. Sample was serially diluted and exactly 0.1ml aliquot of inoculum was aseptically inoculated using the spread plate technique. Plates were incubated at 37^0C for 24 hours. Thereafter, plates were observed for growth of colonies. The colonies were counted to obtain population of bacteria in colony forming unit per gram (cfu/g) of sample. Nmom et al., (2020).

Total Fungal Count (TFC)

The medium Potato Dextrose Agar (PDA) was used for this analysis. The medium was prepared according to manufacturer's direction by weighing and dissolving 39g/l, sterilize by autoclaving at 121^0C for 15 minute, allowed to cool to about 45^0C, amended with 0.1% lactic acid, poured into sterile petri dishes and allowed to solidify. Sample was serially diluted and exactly 0.1ml aliquot of inoculum was aseptically inoculated using the spread plate technique. Plates were incubated at ambient room temperature for 5-7 days. Thereafter, plates were observed for growth of colonies. The colonies were counted to obtain population of fungi in colony forming unit per gram (cfu/g) of sample.

Hydrocarbon Utilizing Bacteria (HUB)

The method described by Chikere et al (2013) was adopted in this study. Mineral Salt Agar (MSA) medium was used to perform this analysis. The medium was formulated using the following salts in g/l; MgSO4; 0.42, KCl; 0.29,KH_2PO4; 1.25, K_2HPO_4; 0.42, NH_4NO_3; 0.83, NaCl; 10.0, Agar; 15, sterilized by autoclaving at 121^0 C for 15 minutes, allowed to cool to about 45^0C,amended with 0.1% lactic acid, poured into sterile petri dishes and allowed to solidify. Exactly 0.1ml aliquot of samples was aseptically inoculated using the spread plate technique. Sterile filter paper was soaked with crude oil and place in the lid of Petri dish. Plates were incubated in inverted position at ambient temperature for 3-5 days. Thereafter, plates were observed for growth of colonies. The colonies were counted to obtain population of hydrocarbon utilizing bacteria in colony forming unit per gram (cfu/g) of sample.

Hydrocarbon Utilizing Fungi

Mineral Salt agar (MSA) was used for this analysis. The medium was prepared by the following salt in g/L: MgSO4; 0.42, KCl; 0.29, KH_2PO_4; 1.25, K_2HPO_4; 0.42, NH_4NO_3; 0.83, NaCl; 10.0, Agar; 15, sterilized by autoclaving at 121^0 C for 15 minutes, allowed to cool to about 45^0C, treated with 0.1% lactic acid to inhibit bacterial growth, poured into sterile petri dishes and allowed to solidify. Exactly 0.1ml aliquots of samples were aseptically inoculated using the spread plate

technique. Sterile filter paper was soaked with crude oil and place in the lid of Petri dish. Plates were incubated in inverted position at ambient temperature for 5-7 days. Thereafter, plates were observed for growth of colonies. The colonies were counted to obtain population of hydrocarbon utilizing fungi in colony forming unit per gram (cfu/g) of sample.

RESULTS AND DISCUSSION

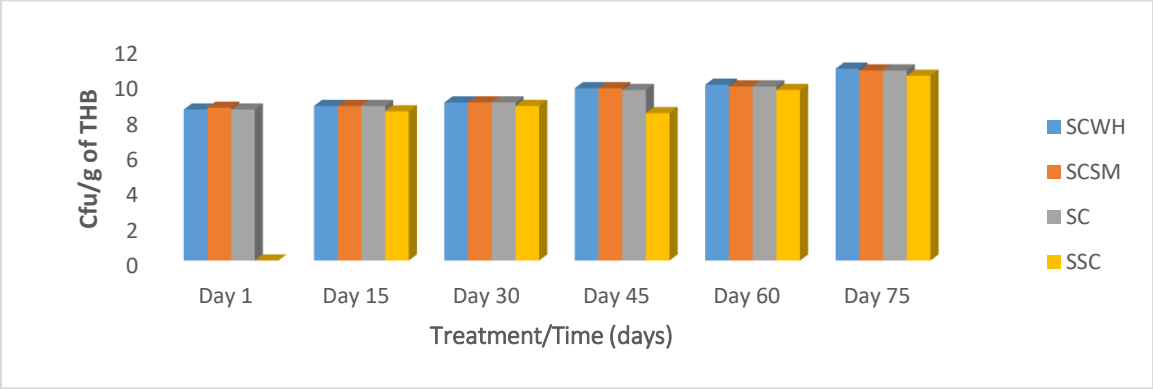

Figure 1: Changes in growth profile of total heterotrophic bacteria (THB) count in the different treatments within the study period.

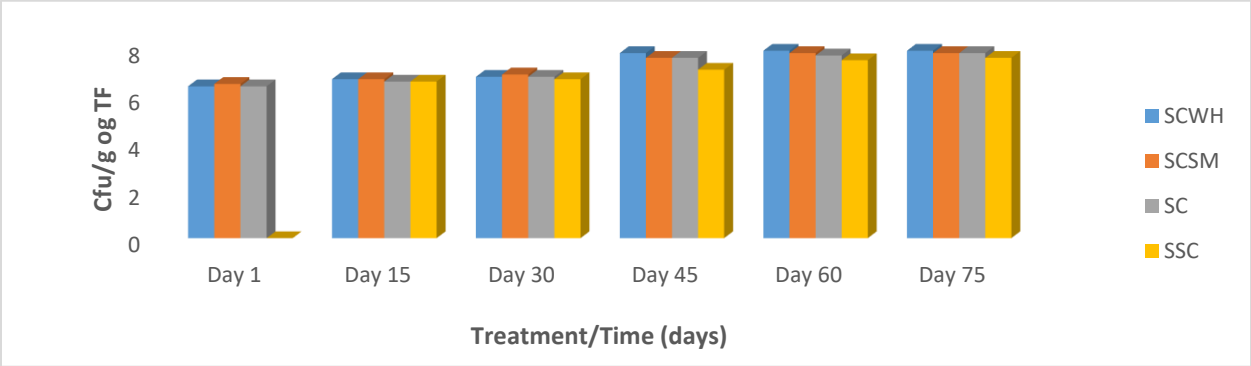

Figure 2: Changes in growth profile of total fungi (TF) count in the different treatments within the study period.

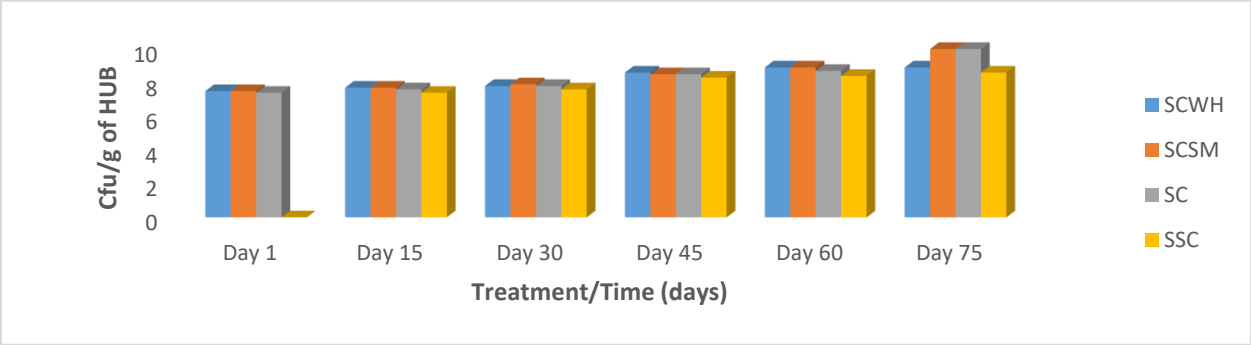

Figure 3: Changes in growth profile of hydrocarbon utilizing bacteria (HUB) count in the different treatments within the study period.

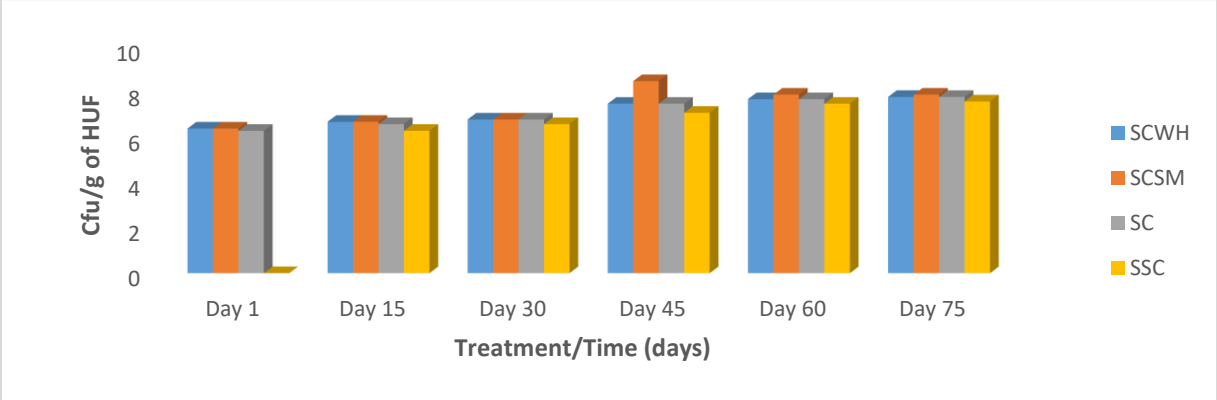

Figure 4: Changes in growth profile of hydrocarbon utilizing fungi (HUF) count in the different treatments within the study period.

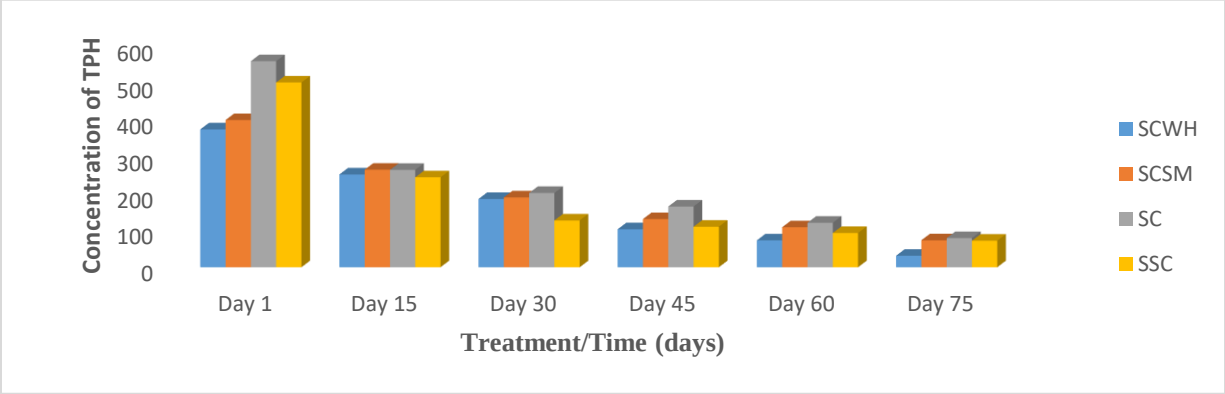

Figure 5: Changes in concentration of total petroleum hydrocarbon (TPH) in the different treatments within the study period.

DISCUSSIONS

There was reasonable increase in the population of microorganisms including Total Heterotrophic Bacteria (THB), Total Fungi (TF), Hydrocarbon Utilizing Bacteria (HUB), and Hydrocarbon Utilizing Fungi (HUF) respectively. The highest population of microorganisms was seen in the treatment with water hyacinth compost within the study period, followed by treatment with spent mushroom substrate. However, treatment with spent mushroom substrate and crude oil alone showed highest population of hydrocarbon utilizing bacteria on day 75. Treatment with sterile soil and crude oil showed zero growth on day one and least population on day 75 compared to other treatments. The bacteria identified in this study include; *Bacillus* sp (19.40%), *Proteus* sp (4.48%), *Pseudomonas* sp (13.43%), *Serratia* sp (4.48%), *Micrococcus* sp. (13.43%), *Arthrobacter* sp. (21.00%), and *Staphylococcus* sp. (23.88%) whereas the fungi include; *Asapergillus* sp. (55.56%), *Saccharomyces* sp. (13.33%), *Penicillium* sp. (4.44%), *Fusarium* sp. (17.78%), and *Rhodotorula* sp. (8.88%). This outcome was consistent with the findings of Omusi et al. (2019) and Simon-Oke et al. (2014) that discovered these bacterial strains. Taken together, these findings support the ability of native microorganisms to efficiently reduce oil pollution. Notably, *Pseudomonas putida* and *Pseudomonas aeruginosa* showed an exceptional capacity to use hydrocarbons as a carbon source and were found to be especially efficient hydrocarbon degraders.

Treatment with water hyacinth compost showed 91.69 % reduction of hydrocarbon pollutant in the crude oil polluted soil while treatment with spent mushroom substrate showed 81.70% reduction of hydrocarbon pollutant in the crude oil polluted soil. These reduction rates are obviously higher in comparison with the treatment with soil + crude (SC); positive control and sterile soil + crude (SSC); negative controls which were 85.87% and 85.64% respectively. this is in contrary to the finding of Ibezute et al. (2024) who proposed that the biological remediation procedure is greatly improved by combining natural sources stimulant substances like water hyacinth with helpful microorganisms strains. This approach is in line with feasible practices by using native resources to improve microbial efficacy, which in turn fosters environmentally helpful remedies to oil pollution.

CONCLUSION

The major findings of this research design indicate that the application of the various plant-based substrates accelerate significantly the reduction of hydrocarbon pollutants as well as remediation time by boosting the potentials of resident hydrocarbon degrading microbes in the soil to multiply and utilize the crude oil pollutants in the soil. The substrates used in this research enhanced microbial growth and metabolism, increasing their population by orders of magnitude thereby,

enhancing their *ex situ* mineralization of organic pollutant in the soil. The research further proves that plant-based substrates which are applied alone or in combination are effective in providing limiting nutrients needed for microbial growth and metabolism of the hydrocarbon pollutants in polluted soil environment. These plant substrates acted as both bulk agents and nutrient suppliers, supporting the growth of indigenous hydrocarbon degrading microorganisms in biodegradation of hydrocarbon pollutants in soil environment. It can be observed from the result obtain in the study that plant biomass presented a cost effective design which reduces the pollutant level in the treatments to a level referred to ‘‘As Low As Reasonable and Practically Possible (ALARPP)’’ which explains that the crude oil pollutant in soil has been reduced to a level where if bioremediation proceeds, it becomes sustainable/economical and favourable for sustainable agriculture. The research has strongly proven that the substrates (plant biomass) used in this research could serve as potential tools for enhanced bioremediation of crude oil polluted soil.

Funding: This work was supported by Tertiary Education Trust Fund (TETFUND), Nigeria [NRF/SETI/NRT/00031]

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