Comparative Study on Bioremediation of Crude Oil Polluted Soil Using Water Hyacinth (*Eichhornia Crassipes*) Compost and Groundnut (*Arachis Hypogea*) Shell.

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Abstract- ***Given the far-reaching effects of oil spills on ecosystems and human health, the cleanup of soil polluted with crude oil is an urgent environmental problem. Crude oil, composed of various hydrocarbons, poses a significant threat to soil quality and biodiversity. The aim of this study is to compare bioremediation study of crude oil polluted soil using water hyacinth (Eichhornia crassipes) compost and groundnut (Arachis hypogea) shell. The standard spread plate technique was used to study the growth dynamics of microorganisms. Bacillus sp (18.40%), Proteus sp (4.48%), Pseudomonas sp (14.43%), 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. (56.56%), Saccharomyces sp. (12.33%), Penicillium sp. (3.44%), Fusarium sp. (18.78%), and Rhodotorula sp. (8.88%), were the fungal isolates identified in the study. TPH reduced by 91.69% in the treatment with Soil +Crude oil + water hyacinth compost (SCWH), 90.37% in the treatment with Soil + Crude oil + Groundnut shell (SCGS), 87.12% in the treatment with Soil Crude, and 85.64% in the treatment with Sterile Soil + Crude oil. 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.***

Indexed Terms- ***Crude Oil, Bioremediation, Ecosystem, Groundnut and Water Hyacinth***

I. INTRODUCTION

Around the world, groundnut (Arachis hypogea), a nutrient-dense leguminous crop, is mostly farmed for its seed and oil. The residue left over after groundnut seeds are extracted from their pods is known as groundnut shells. According to Zheng et al. (2013), this is a common agro-industrial waste product that degrades very slowly in the natural environment. Nonetheless, groundnut shells have a number of useful and bioactive substances that are good for people. Commercial applications include food, feedstock, fertilizer filler, and even bio-filter carriers. However, the majority of abandoned groundnut shells are either buried or burned, polluting the environment. Therefore, in order to achieve zero waste production and turn this otherwise waste product into a useful food ingredient, new technologies must be created. Groundnut shells, a byproduct of the peanut processing industry, have gained attention as a potential bioremediation agent due to their unique properties and environmental compatibility. This agricultural waste material is rich in organic compounds, cellulose, and lignin, making it an

attractive substrate for microbial activity and pollutant degradation (Chinedu., et al. 2018). The use of groundnut shells in bioremediation primarily involves their application as a bioadsorbent or as a source of carbon for microorganisms engaged in pollutant degradation (Singh.,et al. 2020). The porous nature of groundnut shells provides a large surface area, enhancing their adsorption capacity for various contaminants, including heavy metals and organic pollutants (Ahmaruzzaman, 2010). The adsorption process can immobilize pollutants, preventing their migration and facilitating subsequent remediation processes.

In addition to their adsorption capabilities, groundnut shells can serve as a carbon source for microorganisms involved in biodegradation processes. Microorganisms utilized the organic content of groundnut shells as a substrate for metabolic activities, contributing to the breakdown of contaminants into less harmful forms (Wan Zahari., et al. 2019). This dual functionality of groundnut shells - as an adsorbent and a carbon source - makes them a versatile and sustainable option in bioremediation strategies. Research has demonstrated the efficacy of groundnut shells in removing various contaminants from different environmental matrices. For instance, studies have shown successful applications in the removal of heavy metals from wastewater (Khan., et al. 2019) and the enhancement of hydrocarbon degradation in oil-contaminated soils (Agarry., et al. 2015). The ability of groundnut shells to support microbial growth and activity further contributes to their effectiveness in bioremediation applications. Despite the promising attributes of groundnut shells, challenges and considerations exist, such as optimizing conditions for their use, addressing variability in shell composition, and assessing their long-term impact on soil or water ecosystems. Moreover, understanding the specific mechanisms involved in the adsorption and biodegradation processes is essential for tailoring their application to different contaminant types and environmental conditions (Raji et al., 2023).

Composition and Characteristics groundnut shells

The composition and characteristics of groundnut shells play a pivotal role in their suitability as a bioremediation agent. Groundnut shells, also known as peanut shells, are the outer coverings of peanuts and are considered agricultural waste. The following aspects highlight the key composition and characteristics of groundnut shells:

Organic Content: Groundnut shells are rich in organic compounds, including cellulose, hemicellulose, and lignin. These organic constituents make groundnut shells an attractive substrate for microbial activity in bioremediation processes (Chinedu. et al., 2018).

Cellulose and Lignin Content: The cellulose content in groundnut shells contributes to their structural integrity and serves as a source of carbon for microorganisms involved in pollutant degradation. Lignin, another major component, provides rigidity to the shell structure and can contribute to its adsorption properties (Wan. et al., 2019).

Porous Structure: Groundnut shells exhibit a porous structure, resulting in a high surface area. This feature enhances their adsorption capacity, allowing them to effectively trap and immobilize various contaminants, such as heavy metals and organic pollutants, from aqueous solutions or soil matrices (Ahmaruzzaman, 2010).

Surface Area: The large surface area of groundnut shells is crucial for their role as an adsorbent. This feature increases the contact area with pollutants, facilitating adsorption processes and making them effective in removing contaminants from different environmental matrices (Khan. et al., 2019).

Availability and Renewable Nature: Groundnut shells are abundant and readily available as a byproduct of the peanut processing industry. Their use in bioremediation aligns with the principles of sustainability, utilizing agricultural waste for environmental benefit.

Biodegradability: Groundnut shells are biodegradable, and their organic content can serve as a carbon source for microorganisms involved in biodegradation processes. This biodegradability contributes to the overall environmental friendliness of groundnut shells in bioremediation applications (Agarry. et al., 2015).

Mechanisms of Action of groundnut shells in Bioremediation

The use of groundnut shells in bioremediation involves specific mechanisms of action that leverage their unique characteristics. These mechanisms contribute to the effectiveness of groundnut shells as a bioremediation agent, particularly in the context of adsorption and providing a carbon source for microbial activity. The key mechanisms include:

Adsorption Capacity: Groundnut shells possess a porous structure with a large surface area, making them highly effective in adsorbing contaminants from the surrounding environment (Chinedu. et al., 2018). The adsorption mechanism involves the physical or chemical binding of contaminants to the surface of the groundnut shells. This process immobilizes the contaminants, preventing their further movement and facilitating subsequent remediation processes.

Organic Carbon Source for Microbial Growth: The organic composition of groundnut shells, including cellulose and lignin, makes them a suitable carbon source for microbial growth and activity (Wan Zahari. et al., 2019). Microorganisms involved in bioremediation, especially those capable of degrading organic pollutants, utilize the carbon content of groundnut shells as a substrate. This enhances the metabolic activities of the microorganisms and contributes to the breakdown of contaminants into less harmful forms.

Enhancement of Microbial Degradation: Groundnut shells act as a support matrix for microbial communities involved in pollutant degradation. The surface of the shells provides a conducive environment for microbial attachment and growth. This close association between the microbial biomass and the groundnut shells enhances microbial degradation activities, particularly in the case of organic pollutants like hydrocarbons (Agarry. et al., 2015).

Sorption of Heavy Metals: The porous nature of groundnut shells facilitates the sorption of heavy metals from aqueous solutions (Khan. et al., 2019). Heavy metals can be effectively bound to the surfaces of groundnut shells through adsorption, reducing their concentration in water and preventing potential harm to aquatic ecosystems.

Sustainable and Renewable Nature: Groundnut shells offer a sustainable and renewable resource for bioremediation applications. As a byproduct of the peanut processing industry, groundnut shells are readily available and can be utilized without significant environmental impact. Their sustainable nature aligns with the principles of eco-friendly remediation strategies.

Understanding these mechanisms of action is crucial for optimizing the application of groundnut shells in bioremediation processes. It allows for the selection of appropriate conditions and contaminants for which groundnut shells can be most effective, contributing to the development of cost-efficient and sustainable remediation practices.

Water Hyacinth

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).

II. MATERIALS AND METHODS

2.1 *Collection and Preparation of Raw Materials*

Plate 1: Groundnut shell

Plate 2. Water hyacinth compost

Plate 3: pristine soil

Groundnut shell was sourced through a vendor in Abuja Campus, University of Port Harcourt in Obio-Akpor Local Government Area of Rivers State. It was carefully sorted to remove other plant debris and root region of the groundnut itself. The well sorted shell was dried at room temperature for 7-14 days. Thereafter, it will be milled into fine powder before application in 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 inoculums 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.

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; $MgSO_4$; 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,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: $MgSO_4$; 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 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 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.

III. RESULTS AND DISCUSSIONS

3.1 Results

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

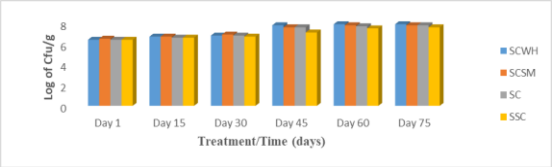

Figure 2: Changes in growth profile of total fungi (TF) count in the different treatments within the study period.

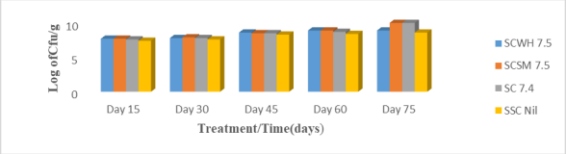

Figure 3: Changes in growth profile of hydrocarbon utilizing bacteria (HUB) count in the different treatments within the study period.

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

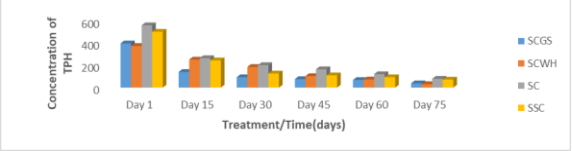

Figure 5: Changes in concentration of total petroleum hydrocarbon (TPH) in the different treatments within the study period.

3.2 DISCUSSIONS

There was a remarkable 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 1 and least population on day 75 compared to other treatments. The bacteria identified in this study include; *Bacillus* sp (18.40%), *Proteus* sp (4.48%), *Pseudomonas* sp (14.43%), *Serratia* sp (2.48%), *Micrococcus* sp. (14.43%), *Arthrobacter* sp. (20.00%), and *Staphylococcus* sp. (25.88%) whereas the fungi include; *Asapergillus* sp. (52.56%), *Saccharomyces* sp. (10.33%), *Penicillium* sp. (7.44%), *Fusarium* sp. (20.78%), 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 groundnut shell 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 proportion is comparable to the results of studies by Sreedhar & Reddy (2019) and Ahamad et al. (2019), which showed that the adsorption of chromium (VI) ions onto plant matter derived from nutshells is most rapid when there is an extra of binding sites and progressively slows down as the proportion of binding sites decreases. This is consistent with the patterns noted by previous researchers who found that the majority for adsorption took place during the first thirty-five minutes after the oil-contaminated location was exposed.

CONCLUSION

The main findings of this research design is that the application of the various plant-based substrates accelerate significantly the reduction of hydrocarbon pollutants concentration 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.

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