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Phytoremediation of Petroleum Hydrocarbon-Contaminated Soil using *Costus afer* Plant

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ABSTRACT
The successful application of the well-known medicinal plant, Costus afer, in the restoration of petroleum hydrocarbon-contaminated soil adds to the list of what the plant can potentially do to rid the environment of harmful chemical compounds to enhance human and environmental health. This study investigated the potential of Costus afer plant at various ages (7, 14, 21, 28, 35, and 42 days old) to restore petroleum hydrocarbon-contaminated soil. The contamination of 48kg of sandy-loam soil was simulated by mixing 0.5, 1.0, and 1.5L of Bonny-Light crude oil with the soil in three separate vessels to achieve conditions of low, medium, and high contamination, respectively. An additional vessel with medium-level contaminated soil served as the control. The Costus afer plants were nursed and transplanted at the stated ages to each vessel except the control. Controlled irrigation was applied, and the setups were housed to shield them from rainfall. After 90 days of treatment, results showed that the 7 days old Costus afer plants produced the highest amounts of total petroleum hydrocarbon (TPH) reduction of 97.42, 94.64, and 95.12% in the soil with low, medium, and high contamination, respectively. Furthermore, the sequence of TPH reduction by the plants was 14 days old, 21 days old, 28 days old, 35 days old, and 42 days old. Thus, in addition to its medicinal value, Costus afer plant also has the potential to decontaminate petroleum hydrocarbon-contaminated soils.

KEYWORDS: Bioremediation; Crude Oil Spill; Environmental Engineering; Land Contamination; Costus *afer*

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1. INTRODUCTION

Soil is one of the most important natural resources on earth (Bhattacharya *et al*., 2015). It provides a growing medium for plant roots, minerals and nutrients, exchanges oxygen and gases, protects against erosion, and speeds up the natural decomposition process of organic matter. Thus, healthy soil is crucial for the well-being of humans, animals, and plants. Soil, in most parts of the world, is polluted including the Niger Delta soil in Nigeria. petroleum hydrocarbon contaminated soil poses a significant concern to humans and to the environment worldwide. Petroleum hydrocarbon fills soil pore spaces and inhibits effective soil aeration; thereby, suffocating plants that need effective air circulation in the soil for their growth and development. It also strips the soil of its natural nutrients and inhibits plants from absorbing appropriate nutrients, which eventually leading to plant mortality, depletes oxygen in the soil, destroys soil microorganisms useful for plant growth, and increases soil temperature, toxicity as well as reduces soil fertility (Igoni, 2018).

Oil spills in Nigeria are caused by the corrosion of pipelines due to a lack of adequate maintenance and vandalization of pipelines (Okparanma, 2013; NNPC, 2021). Petroleum contamination of soil is a common type of soil contamination within the region (Fayiga *et al*., 2018). Petroleum consists of thousands of organic materials, most of which are hazardous hydrophobic compounds. The elemental composition of petroleum is oxygen (1-1.5%), Nitrogen (10 – 14%), carbon (85- 90%), Sulphur

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(0.2-3%), hydrogen (<0.1-2) and metals (<1) (Sayeed *et al*.,2021). Depending on the source of the Petroleum, petroleum hydrocarbons composed about 50–98% of crude oil and are regarded as a major component. (Al-Dhabaan, 2019). Crude oil comprises of four major compounds: saturates, resins, aromatics and asphaltenes (Al-Hawash *et al*., 2018). These components can alter the soil's physical, chemical, and biological quality and when exposed to it, they can cause carcinogenic and immunotoxic effects, thereby posing a serious threat to human and environmental health (Sales da Silva *et al*. 2020).

Due to the impact of petroleum hydrocarbon in the soil, challenges of hydrocarbons in the environment have grown immensely. Total petroleum hydrocarbons are of particular importance because of the accumulation of these contaminants in the soil, might pose a serious threat to humans and other living organisms through different pathways (Denys *et al*., 2006). Decontamination of hydrocarbon-polluted soils has long been a global problem that is recently being tackled in various ways. For instance, physicochemical techniques including incineration, disposal in landfills, excavation, thermal desorption, soil flushing, soil washing, soil vapour extraction, stabilization/solidification, etc. have generally been used (Ossai *et al*., 2020; Vidonish *et al*., 2016). However, these physicochemical methods are quite expensive and are not environmentally friendly.

Currently, a spectrum of biological treatment technologies for hydrocarbon-contaminated soils has been demonstrated *et al* to be effective (Wang *et al*., 2019; Guarino., 2017; Ossai *et al*., 2020, Okparanma *et al*., 2022). Biological treatment, also known as bioremediation, entails the use of microorganisms to break down pollutants into non-toxic forms (Riser-Roberts, 1998). It has been canvassed and adopted over chemical technologies because it is relatively cheap and ecologically friendly (Muttaleb & Ali, 2022). Some well-known biological treatment technologies include (but are not limited to) phytoremediation, bioaugmentation, and biostimulation.

Phytoremediation is a low-cost and environmentally friendly technology that uses plants to remove a wide range of organics and inorganics from the soil (Singh & Singh, 2017). The contaminants are removed by one or more of many phytoremediation processes including phytodegradation, phytostabilization, phytovolatilization, and phytoextraction (Thijs *et al*., 2017). In this study, the phytoremediation strategy adopted was phytostabilization, which involves the use of contaminant-tolerant plant species to immobilize pollutants in the soil and decrease their bioavailability. This prevents the migration of pollutants into the ecosystem and reduces the likelihood of the pollutants finding their way into the food chain (Wong, 2003; Marques *et al*., 2009). Kathi and Khan (2011) suggested that exploiting natural biodiversity by identifying appropriate native species that grow in contaminated soil and recognizing the mechanisms involved in soil-plant interactions may broaden the scope of restoration efforts.

Some plants have been reported to have the potential to facilitate the remediation of petroleum hydrocarbon-contaminated sites such as spear grass, guinea grass, elephant grass, and gamba grass (Kogbara *et al.,* 2018), corn and elephant grass (Ayotamuno *et al*., 2006), etc. These studies showed that some of these plants have more potential to remediate contaminated soils than others. Studies also have shown that plant type and age are among the factors that affect the phytoremediation of contaminated soils, which if appropriately selected would efficiently remediate the contaminated soil as well as reduce cost. Therefore, there is a need for bioremediation studies to consider other plant

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types at different ages to ascertain which plants and at what age the plants are most effective in remediating contaminated soils.

Currently, no studies have reported the use of *Costus afer* plant in the remediation of petroleum hydrocarbon-contaminated sites. *Costus afer* plant (Plate 1) commonly called bush sugarcane or monkey sugarcane (Nyananyo, 2006), is a monocot belonging to the family *Zingiberaceae*, which is relatively a tall, herbaceous unbranched tropical plant.

Plate 1: *Costus afer* plant

It is typically found in West and Tropical Africa's damp or shady woods (Iwu, 2009). The native plant is a dominant crop in Nigeria's Niger Delta region. It grows like a weed, despite its usefulness in the pharmaceutical industry. The plant is still in great quantity in the region and is yet to be utilized for the remediation of crude oil-contaminated sites. The purpose of this study was to examine the potential of *Costus afer* plants at different ages to remove TPH in soils contaminated with crude oil.

2. MATERIALS AND METHODS

2.1 The Study Area

The research was carried out at the Rivers State Institute of Agricultural Research and Training, Rivers State University, Port-Harcourt, Nigeria (latitude 4.800482°E and longitude 6.97702°N). According to the United States Department of Agriculture (USDA) soil taxonomic order, the soil in the area is predominantly Oxisols (Okparanma, 2013). Rivers State (Figure 1) is characterized by tropical rainforest vegetation, with annual rainfall ranging from 2000 to 2484mm, with 70% falling between the months of May and August with an average temperature of 27°C (Ayotamuno *et al.*, 2006; Fubara-Manuel *et al.*, 2021).

Figure 1: The Study Area, Rivers State, in the Niger Delta region of Nigeria.

2.2 Experimental Design and Oil Spill Simulation

The experimental design used in this study was the group-balanced block design (GBBD). For oil spill simulation, the procedure used by Ayotamuno *et al.* (2006) was adopted to arrive at the three working concentrations (low, medium, and high) of crude oil in the soil used in this study. To do this, about 48kg of sandy-loam soil was placed in four separate vessels. Then, three of the vessels were contaminated with 0.5, 1.0, and 1.5 litres of Bonny-Light crude oil, in turn, to simulate conditions of low-, medium-, and high-level contaminations, respectively. The medium-level contamination was duplicated to create a fourth vessels, which was used as the control. The two main variables were crude oil concentration (C) as factor A having 3 levels

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including low concentration (C_1), medium concentration (C_2), and high concentration (C_3); and age of *Costus afer* plant (T) as factor B with 6 levels including 7 days old (T_1), 14 days old (T_2), 21 days old (T_3), 28 days old (T_4), 35 days old (T_5), and 42 days old (T_6).

2.3 Experimental Setup

The artificially contaminated soil used as planting medium was placed in wide-mouth black plastic basins (vessels) with a depth of 0.3m and a top diameter of 0.5m as shown in Plate 2. The vessels were kept in an open barn to shield them from direct rainfall for moisture control. Before transplanting the nursed *Costus afer* plants, the contaminated soil in the vessels was allowed for a three-day incubation period. Each vessel was irrigated with 0.5L of water at three days intervals until the cessation of remediation. This water application rate was in line with the application rates used by Ayotamuno *et al.* (2010), who showed its effectiveness in the remediation of crude oil-polluted soils. *Costus afer* plants were nursed for 7, 14, 21, 28, 35, and 42 days. Thereafter, they were transplanted to the planting medium. The experimental layout is shown in Plate 2.

Plate 2: Experimental Setup for the Phytoremediation of Crude Oil-Contaminated Soil using *Costu afer* Plant at Different Ages.

2.4 Sampling and Analytical Methods

In this study, we did not consider the determination of the TPH level in the crude oil used for soil pollution simulation because it was of no consequence since the starting concentration of TPH in the treatment process was identified in the polluted soil and not the crude oil. Besides, the characteristics of diverse types of crude oil have been widely documented and are readily available in their respective material safety data sheets. Prior to and after artificial contamination, composite soil samples were collected for physicochemical analysis. The physicochemical characteristics of the uncontaminated soil tested were pH, electrical conductivity (EC), moisture content (MC), particle size distribution (PSD), organic matter (OM), organic carbon (OC), and TPH. The TPH of the contaminated soil was also determined at intervals of 30 days for a period of 90. A handheld H198331 multimeter (Hanna Instruments, USA) was used to conduct an in-situ measurement for pH and EC. Soil MC was determined by the 24-hour oven-drying method. The hydrometer method was used to determine the PSD while the soil texture was determined by the USDA soil textural classification scheme using TAL® for Windows Version 4.2. The OM and OC were determined by the Walkley-Black combustion method. TPH compounds were extracted by the 5-hour sonication water bath method using dichloromethane as the extraction

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solvent. Then, TPH was quantified by the external standard method, according to USEPA (1996) method 8015B using a gas chromatograph coupled to a flame ionization detector (FID). The percentage of TPH removal was deduced using equation 1.

$$TPH\ removal\ (\%) = \frac{IC - FC}{IC} \times 100 \qquad (1)$$

Where, IC = initial concentration of TPH (mg/kg), and FC= final concentration of TPH (mg/kg).

2.5 Statistical Analysis

The standard deviation, mean, and standard error were determined using STDEV, AVERAGE, and Standard Error functions, respectively in Excel-365 (Microsoft Inc., USA). Simple percentages were also determined. Data were analyzed using the one-way analysis of variance (ANOVA) following the method described by Gomez and Gomez (1983) to determine if there were statistically significant differences within and among treatments at the 5 and 1% significance levels based on the *F*-test. Differences were considered significant if the calculated *F*-value was greater than or equal to the tabular *F*-value, and nonsignificant if otherwise.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of the Uncontaminated Soil

Table 1 shows the key properties of the pristine soil used as the planting medium. The uncontaminated soil is made up of 27.4% silt, 60% sand, and 12.6% clay; and is classified as sandy loam as shown in Figure 2. The soil was slightly acidic with an EC of 15.6 µS/cm. TPH was below the limit of quantitation in Nigerian soils.

Table 1: Physicochemical Properties of uncontaminated soil

Soil Characteristics	Value
pH	5.86
Moisture content (%)	12.08
Electrical conductivity (µS/cm)	15.6
Organic matter (%)	0.98
Organic carbon (%)	0.57
TPH (mg/kg)	< 0.02
Particle Size Distribution	
Silt (%)	27.4
Sand (%)	60
Clay (%)	12.6
Textural Class	Sandy-Loam

TPH, Total Petroleum Hydrocarbon

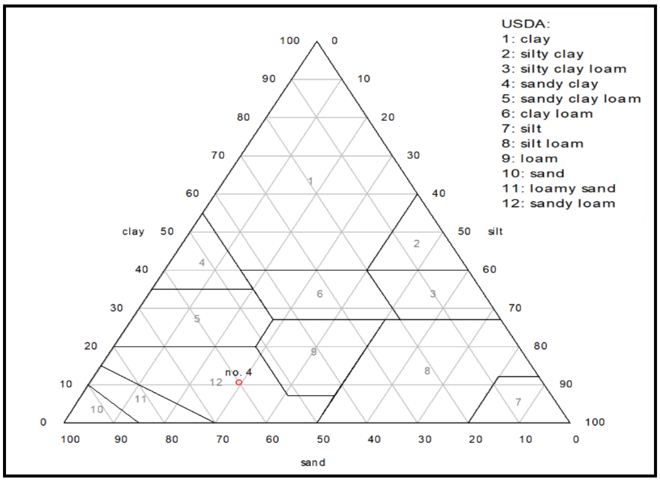

Figure 2: Soil Textural Triangle Showing Sandy Loam (No. 12) as the texture of the soil used in this study based on the USDA Soil Textural Classification Scheme as Determined using TAL® for Windows v. 4.2 (Christopher Tech Boon Sung, China).

3. 2 TPH Characteristics of the Untreated Crude Oil-Contaminated Soil

Figure 3 shows the TPH characteristics of the untreated petroleum hydrocarbon-contaminated soil. The crude oil-contaminated sandy-loam soil contains TPH, as corroborated in several pieces

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of literature including Okparanma & Mouazen (2013). It was also observed in Figure 3 that the TPH content of the untreated soils including the control was well above the 5,000mg/kg intervention value spelt out by DPR (2018), suggesting that the soil is unsafe for agricultural purposes. Thus, there is a high priority for remediation before the soil can be used for agricultural or other purposes.

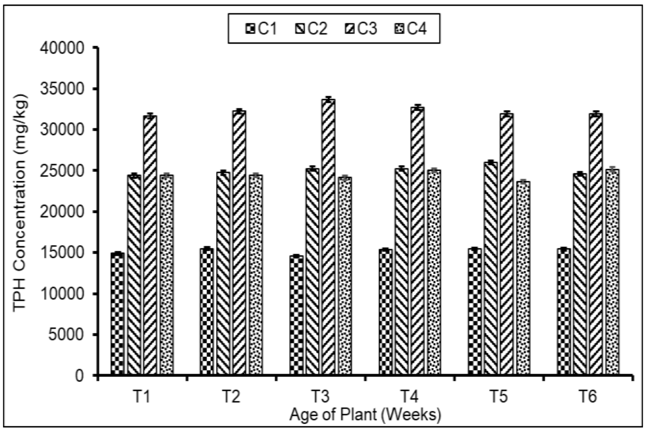

Figure 3: TPH Concentrations in the Polluted Sandy-Loam Soil Before Commencement of Treatments. [T1 = 1 week old, T2 = 2 weeks old, T3 = 3 weeks old, T4 = 4 weeks old, T5 = 5 weeks old, and T6 = 6 weeks old; C1, C2, and C3 = low, medium, and high contaminated soil with plants, respectively and C4 = medium contaminated soil without plants (control); Error bars on charts represent standard errors].

3.3 TPH Degradation Over Time

Figure 4 shows the residual TPH concentrations in the polluted sandy-loam soil after the stated days of remediation. As evident in Figure 4, after 30 days of treatment with the *Costus afer* plant, there were varying levels of TPH reduction across the treatments. The drop in TPH concentration in the low-level contaminated soil (C_1) treated with the 7 days old plants ranged from 3,008–3,516mg/kg. Although these values were above the remediation target value of 50mg/kg, the 7 days old *Costus afer* plant was able to reduce the TPH level to below the DPR (2018) intervention value of 5,000mg/kg. This means that apart from agricultural purposes the remediated land could still be put to other forms of use like building construction, etc. The degradation of TPH increased with time, proceeding at a fast rate in the first 30 days, and becoming slower afterwards. This agrees with the findings of Okparanma *et al*. (2017; 2021; 2022). The control was far beyond the DPR (2018) intervention value.

At 30 days after planting, *Costus afer* plants were characterized by leaf chlorosis (yellowing of leaves), but as time progressed the leaf discolouration decreased substantially for all treatments. This observation, according to some studies, was typical of the adaptation mechanism of some plants to crude oil pollution (Ayotamuno *et al*., 2007; Schwab *et al*., 1999; and Witse *et al*., 1998). This was observed more in the older plants than the younger plants in the medium- and high-contaminated soils. Leaf chlorosis may be a result of stress-induced colour changes because of nutrient imbalance caused by hydrocarbon contamination (Lemus, 2012).

At 90 days after planting, further decrease in the TPH concentration ranged from 383.67–1,114mg/kg for the low-contaminated soils for which 97.42, 95.34, 93.4, 93.16, 92.84, and 92.78% TPH reduction was recorded for T_1, T_2, T_3, T_4, T_5, and T_6, respectively. A similar trend was observed for other contamination levels. Overall, the TPH concentration in all the treatments dropped below the DPR (2018) intervention value of 5,000mg/kg but was above the remediation target value of 50mg/kg. However, the TPH reduction level in the control vessel was very low (10.71-15.04%) for all the treatments.

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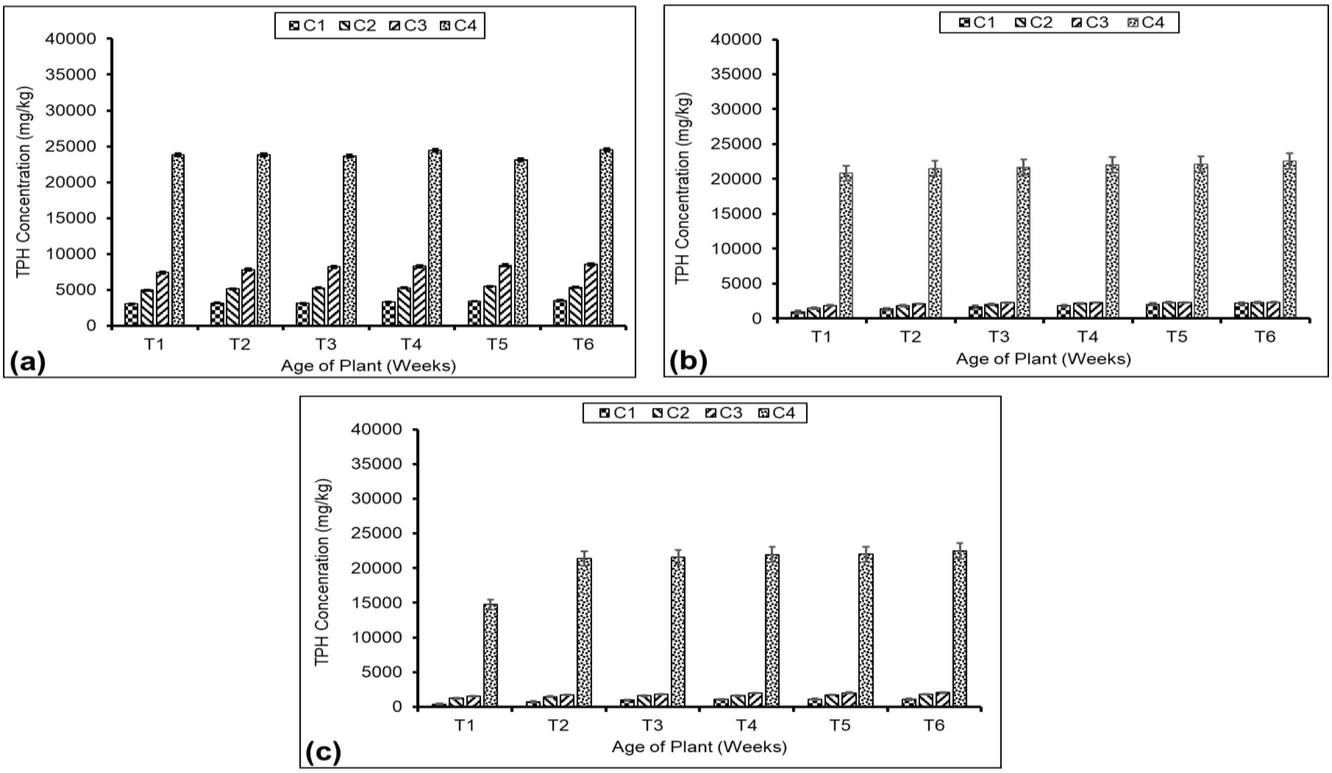

Figure 4: Residual TPH Concentrations in Polluted Sandy-Loam Soil after: (a) 30 days (b) 60 days, and (c) 90 days of Treatment with *Costus Afer* Plant at Different Plant Ages. [T1 = 1 week old, T2 = 2 weeks old, T3 = 3 weeks old, T4 = 4 weeks old, T5 = 5 weeks old, and T6 = 6 weeks old; C1, C2, and C3 = low, medium, and high contaminated soil with plants, respectively and C4 = medium contaminated soil without plants (control); Error bars on charts represent standard errors].

Figure 5 compares the performance (in terms of TPH reduction) of the *Costus afer* plant at different ages over time in the crude oil-contaminated sandy-loam soil. It is evident from Figure 5 that the TPH reduction was highest in the soil treated with the 7 days (1 week) old *Costus afer* plants (T_1), followed by T_2, T_3, T_4, T_5, and T_6; and these occurred after 90 days of treatment. The same trend was observed in the medium- and high-level contaminated soils, suggesting that the 1-week-old *Costus afer* plants outperformed the other ages in terms of TPH reduction (Figure 5). These findings agree with Efe and Okpali (2012), and McClutchen and Schnoor (2003), who observed that phytoremediation can be utilized efficiently to handle petroleum hydrocarbon-contaminated soil.

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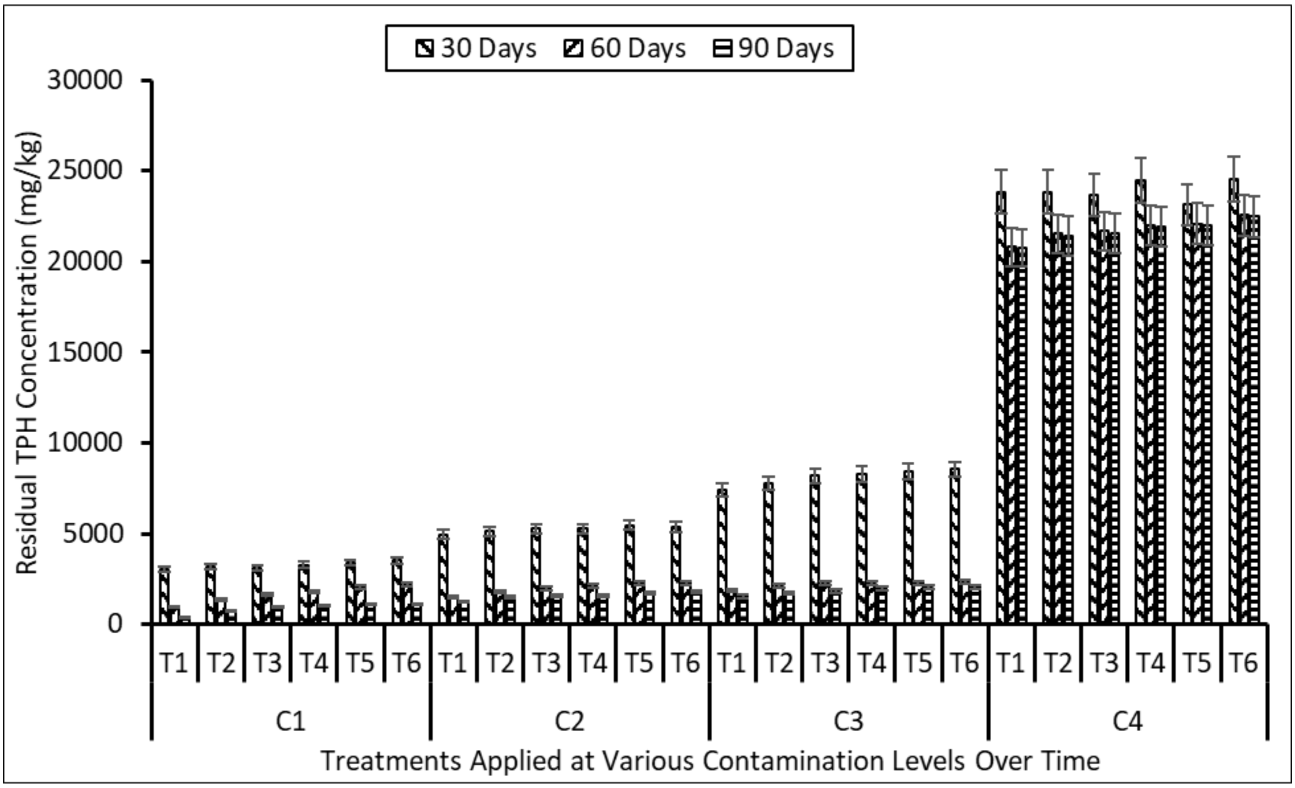

Figure 5: Comparison of the Performance (in terms of TPH reduction) of *Costus afer* Plant at Different Ages Over Time in Crude Oil-Contaminated Sandy-Loam Soil. [T1 = 1 week old, T2 = 2 weeks old, T3 = 3 weeks old, T4 = 4 weeks old, T5 = 5 weeks old, and T6 = 6 weeks old Plants; C1 = Low Contamination Level; C2: Medium Contamination Level; C3: High Contamination Level; C4: Medium Contamination Without Treatment (Control); Error bars on chart are standard errors].

In comparison with other plants, results obtained in this study revealed that the phytoremediation potential of *Costus afer* after 90 days of treatment for the various treatments at all levels of contamination except the control (C_4) exceeded those of Barley, Guinea grass, and Purple nutsedge plants. Barley (*Hordeum Vulgare*) plant reduced TPH in a petroleum hydrocarbon-contaminated soil by 83% from an initial concentration of 75,000mg/kg within a remediation period of 90 days (Azeez & Qasim, 2021). In the same vein, the Guinea grass (*Megathyrsus maximus*) plant reduced TPH in a petroleum hydrocarbon-contaminated soil by 58% from an initial concentration of 4,805mg/kg after 90 days of treatment (Kogbara *et al*., 2018). Again, the Purple nutsedge (*Cyperus rotundus*) plant remediated petroleum hydrocarbon-contaminated soil by 66% from an initial concentration of 5,726.34mg/kg at 90 days remediation period (Nwaichi *et al*., 2021).

It is important to note that the ability of *Costus afer* to thrive and grow in crude oil-contaminated soil may be attributed to its nitrogen-fixing ability. It could also be a result of the development of an extensive fibrous root system by the *Costus afer* plant, which may be an adaptation to enhance its tolerance and survival strategies in dealing with the water

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stress caused by crude oil (Okonwu *et al*., 2013). The variations in the reduction of TPH agrees with the findings of Abdel-Ralum *et al.* (1983) and Gonzaga *et al.* (2007), suggesting that the influence of microorganism varies with plant age as well as plant type. It has been reported that remediation of Arsenic levels is higher in younger plants than in older plants, probably due to younger plants' stronger metabolic activity (Gonzaga *et al*., 2004), which corroborates the findings of this study that 7 days old *Costus afer* plants had higher phytoremediation potential than the older *Costus afer* plants. As there was a gradual reduction of TPH in the reactor without treatment (C_4) across all levels of crude oil contamination, this natural attenuation may be occasioned by atmospheric influence (Efe & Okpali, 2012). The ANOVA result (Table not shown) showed that there was a significant difference (calculated *F*-value > tabular *F*-value) in the treatment means at the 5% significance level. Thus, it can be concluded with 95% confidence that the observed difference in the treatment means was because of the treatment applied.

4. CONCLUSION

The phytoremediation potential of *Costus afer* plants at different ages over a period of 90 days was investigated with a view to ascertaining their suitability for decontaminating petroleum hydrocarbon-contaminated soils. Results showed that the 7 days old *Costus afer* plant was the most suitable for remediating low-, medium-, and high-level crude oil contaminations in soils, accounting for a TPH reduction of 97.42, 94.64, and 95.12%, respectively. The sequence of TPH reduction by the plants was 14 days old > 21 days old > 28 days old > 35 days old > 42 days old. Thus, the *Costus afer* plant has the potential to decontaminate petroleum hydrocarbon-contaminated soils. However, it is recommended that future studies should consider the effect of hydrocarbon-utilizing bacteria in crude oil-contaminated soil on TPH reduction.

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