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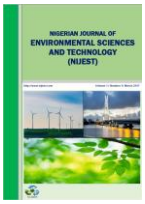

Elephant Grass (*Pennisetum Purpureum*) Mediated Phytoremediation of Crude Oil-Contaminated Soil

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ABSTRACT

Phytoremediation is an economic and environmentally friendly method for the remediation of hazardous crude oil contaminated soils. In this study, phytoremediation of crude oil contaminated soils by elephant grass (pennisetum purpureum) was investigated over a 40-day period. Grass clumps were harvested and transplanted into plastic buckets filled with 1kg of soil contaminated with 30 ml and 40 ml of crude oil and an uncontaminated control sample. An additional control sample was contaminated with 30 ml of crude oil with no elephant grass. The samples were analyzed periodically for changes in pH, total hydrocarbon content, total viable bacterial count, and total fungal count. The pH of the soil samples generally ranged from 5.26 to 7.85. After 40 days of treatment, the total hydrocarbon content decreased from 320 mg/kg to 38 mg/kg and from 590 mg/kg to 46 mg/kg in samples contaminated with 30 ml and 40 ml of crude oil respectively. Plant growth was uninhibited in contaminated and control samples as the heights increased by 34.5-42.8 cm. The results of the study further demonstrate the phytoremediation capabilities and tolerance of elephant grass in crude-oil contaminated microcosms.

Keywords: Phytoremediation, Elephant grass, Crude oil, Hydrocarbon, Contaminated soil

1.0. Introduction

In oil-producing developing countries, environmental degradation is a major problem due to accidental crude oil spillages resulting in the pollution of vast amounts of agricultural land and aquatic systems (Ugochukwu and Ertel, 2008). Adverse effects of crude oil contamination include, loss of fertile soils, air and water pollution, destruction of ecosystems, plant and animal poisoning and the potential ingestion and subsequent risk to human health (Abii and Nwosu, 2009; McGuinness and Dowling, 2009). Due to their chemical structure, organic compounds such as petroleum hydrocarbons are resistant to natural breakdown processes and persist for years when released into the environment (McGuinness and Dowling, 2009). Crude oil spillages on land may be due to land disposal of wastes from refineries, leaking oil storage facilities, accidental or criminal damage of pipelines, corrosion of old pipelines and oil tanker accidents (Fine *et al*., 1997; Abii and Nwosu, 2009; Izinyon and Seghosime, 2013). The presence of crude oil in soil pore spaces causes depletion of oxygen reserves and hinders soil-atmosphere gas exchange (Ayotamuno *et al*., 2006). Volatilization of light hydrocarbon fractions from contaminated soils releases substantial amounts of potentially carcinogenic compounds which are detrimental to human health. Petroleum hydrocarbon contamination has also been reported to affect plants by retarding seed germination and reducing shoot height, stem density, photosynthetic rate and biomass yield (Lin and Mendelssohn, 1996; Fine *et al*., 1997).

Conventionally, physicochemical treatment methods such as thermal desorption, incineration, solvent extraction, landfilling, etc. have being employed in the remediation of hydrocarbon-contaminated soils (Jain *et al*., 2011). However, the high costs and negative environmental impacts of these methods have resulted in heightened interest in exploring the potentials of phytoremediation as a relatively less expensive alternative (Frick *et al*., 1999; Nedunuri *et al*., 2000). Phytoremediation involves the use of

plants and their associated microbes in the extraction, sequestration and degradation of contaminants in aqueous and solid phases. Studies have shown that certain plants have the ability to clean-up several pollutants including metals, pesticides and hydrocarbons (Frick *et al*., 1999; Nedunuri *et al*., 2000; Merkl *et al*., 2005). This remediation method is environmentally friendly, and the soil structure is preserved. It is particularly suited to tropical climates due to the inherent favorable conditions for microbial growth and activity, nutrient availability and biomass production (Merkl *et al*., 2005). Several plants including *tithonian diversifolia*, cyperus rotundus, *phyllantus amarus*, *centrosena pubescen*, *ipomoea batatas*, *pennisetum pupureum*, etc. have been investigated for the remediation of soils contaminated with various petroleum hydrocarbon fractions with findings published in the literature (Ayotamuno *et al*., 2006; Ogbo *et al*., 2009; Nwaichi and Onyeike, 2011; Izinyon and Seghosime, 2013; Udo-Inyang *et al*., 2013; Efe and Elenwo, 2014; Omovbude and Udensi, 2016).
In this study, phytoremediation of crude oil- contaminated soil using locally abundant elephant grass (pennisetum purpureum) was investigated. The specific objectives were to evaluate the phytoremediation capabilities of the plant within the study period and examine the impact on plant growth.

2.0. Materials and Methods

2.1. Collection and preparation of soil samples

Soil samples and elephant grass used in this study was obtained from a farmland close to the University of Benin, Nigeria. Bonny light crude oil was collected from a company in Port Harcourt, Nigeria. The pH, moisture content, total hydrocarbon content (THC) was determined according to procedures described in Adesodun and Mbagwu, 2008 and Akpe *et al*., 2015. The pH of the soil was determined using a pH meter immersed in soil-water slurry consisting of air-dried and sieved soil mixed at 1 g/ml and left to equilibrate for 30 minutes. The moisture content of the soil was determined using the gravimetric method. The soil sample was oven-dried at 105°C and the moisture content was calculated as a percentage of the oven-dried weight. THC was determined by hexane extraction. 5g of soil was mixed with 23 ml of hexane for 20 minutes and filtered. The absorbance of dilutions of the sample was measured using a spectrophotometer and the concentration of THC was determined based on standard curves for petroleum fractions. The total viable bacterial count and total fungal count were estimated using the standard spread plate technique (APHA, 1992). Serial dilutions of a soil suspension (1 g soil/10 ml distilled water) were spread on the surface of nutrient agar and incubated at 35˚C for 5 days for bacterial isolation and potato dextrose agar incubated at ambient temperature for 7 days for fungal isolation.

2.2. Experimental Set-up

Phytoremediation studies were conducted over a 40-day period in four plastic buckets filled with 1 kg of soil (Figure 1). Two buckets were contaminated with 30ml and 40ml of crude oil respectively and treated with elephant grass. Two buckets served as controls (one bucket was contaminated with 30ml of crude oil with no elephant grass, while the second bucket contained uncontaminated soil with elephant grass). The samples are described in Table 1.

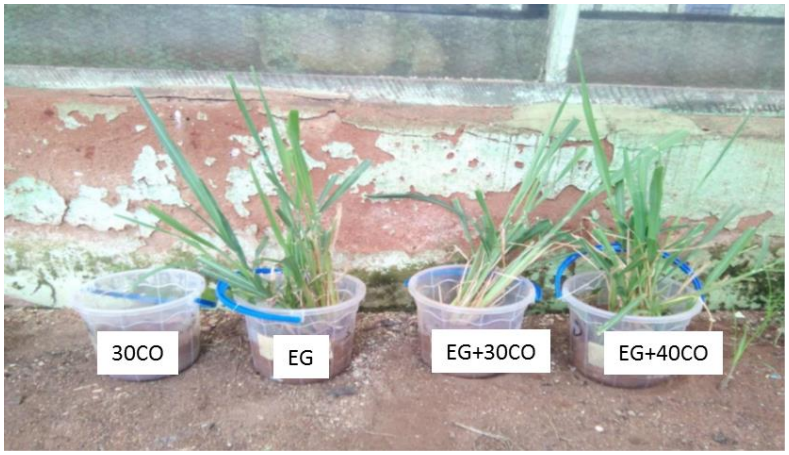

Figure 1: Experimental set-up showing contaminated samples and controls

Table 1: Description of experimental samples

Sample	Description
EG+30CO	Elephant grass planted in soil contaminated with 30 ml of crude oil
EG+40CO	Elephant grass planted in soil contaminated with 40 ml crude oil
30CO	Control (Unplanted soil contaminated with 30 ml crude oil)
EG	Control (Elephant grass planted in uncontaminated soil)

The plants were watered daily and soil samples were collected at 10-day intervals for 40 days. The samples were analyzed for changes in pH, total hydrocarbon content, total viable bacterial count and total fungal count. Plant growth was assessed at 5-day intervals by direct measurement using a meter rule.

3.0. Results and Discussion

3.1. Crude oil removal performance

The analysis of a representative soil sample prior to contamination and treatment revealed that the soil moisture content was 15.4%, pH was 6.80, total hydrocarbon content was < 0.001 mg/kg, total viable bacterial count was 188cfu/g and the total fungal count was 212 cfu/g.

The residual THC concentrations in the contaminated samples and control were determined as shown in Figure 2 and Table 2. It can be observed that the total hydrocarbon content decreased from 320 to 38 mg/kg in EG+30CO, 590 to 46 mg/kg in EG+40CO and 360 to 142 mg/kg in the contaminated control sample (30CO) after 40 days of treatment. The reduction in THC in the soils undergoing treatment is in agreement with the findings from similar studies (Ayotamuno *et al*., 2006; Udo-Inyang *et al*., 2013).

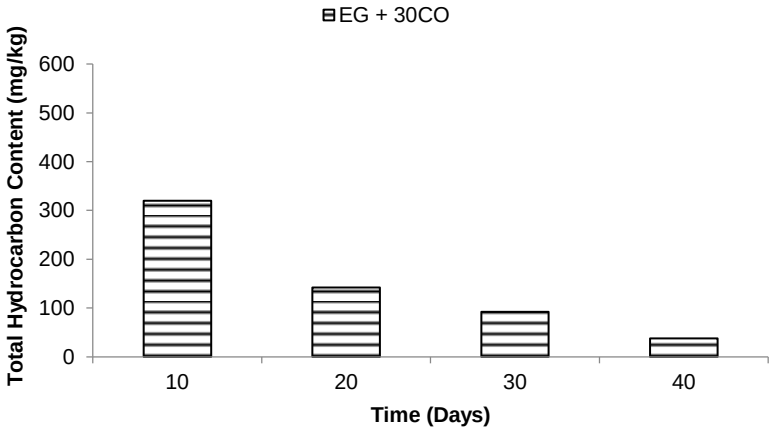

Figure 2a: Reduction in total hydrocarbon content (sample contaminated with 30 ml of crude oil)

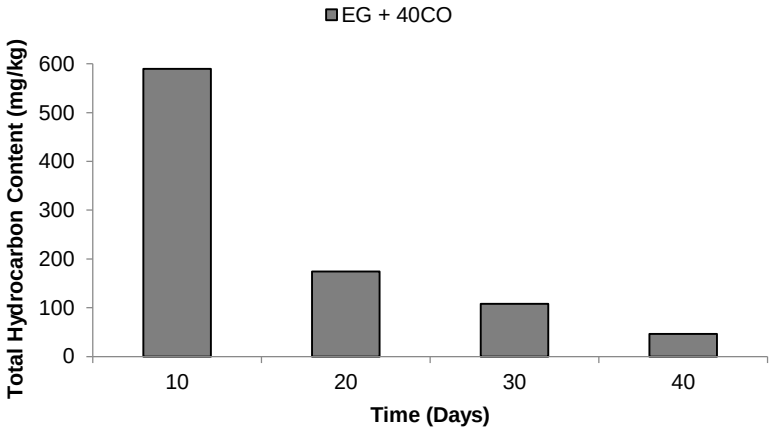

Figure 2b: Reduction in total hydrocarbon content (sample contaminated with 40 ml of crude oil)

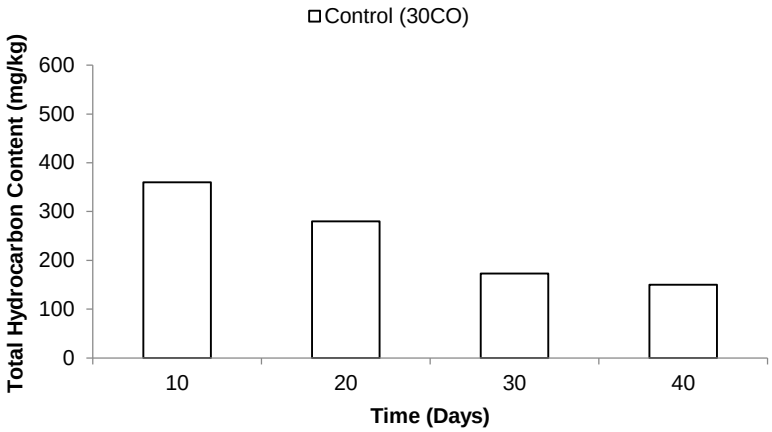

Figure 2c: Reduction in total hydrocarbon content (sample contaminated with 30ml of crude oil)

Phytoremediation of petroleum hydrocarbons may be due to the combined mechanisms of phytodegradation of complex organic molecules taken up by the plant, rhizodegradation involving plant associated bacteria and fungi in the root zone (rhizosphere) and phytovolatilization of the volatile organic fractions (Etim, 2012; Omovbude and Udensi, 2016). The reduction in THC in 30CO can be attributed to biodegradation by some microbial species present in the soil as reported in other studies (Van Hamme *et al*., 2003; Akpe *et al*., 2015). The soil pH ranged from 5.26 to 7.85, with a general decrease observed after 40 days of treatment (Table 2). This suggests the possible presence of sulfur forming minerals which produce acidic conditions on exposure to air or an inherent low buffering capacity (USDA, 1998). The observed increase in microbial counts, particularly in the remediated soil samples indicate the importance of microbial activity and rhizodegradation occurring in the root zone.

Table 2: Characteristics of contaminated soil and control samples

	EG+30CO	EG+40CO	Control (30CO)	Control (EG)
Time (Days)	pH			
10	5.93	7.11	6.90	6.53
20	6.88	6.80	6.82	6.71
30	7.30	7.14	7.05	7.65
40	5.80	5.80	5.53	5.26
	Total Hydrocarbon Content (mg/kg)			
10	320	590	360	<0.001
20	142	174	280	<0.001
30	92	108	173	<0.001
40	38	46	142	<0.001
	Total Viable Bacterial Count (cfu/g)			
10	192	168	107	252
20	206	180	124	274
30	248	290	352	308
40	414	432	514	486
	Total Fungal Count (cfu/g)			
10	160	124	86	234
20	178	152	106	246
30	232	258	188	352
40	362	384	438	410

3.2. Impact of crude oil contamination on plant growth

The growth of plants in contaminated soils and controls was evaluated by direct measurements. The plants grew steadily, with no evidence of inhibition due to crude oil contamination (Figure 3). The average plant heights increased by 33.7 cm, 42.8 cm and 34.5 cm in EG+30CO, EG+40CO and EG respectively. This resilience suggests the ability of the plant to grow in contaminated environments and the appropriateness of the plant for phytoremediation applications (Wenzel *et al*., 1999; Izinyon and Seghosime, 2013).

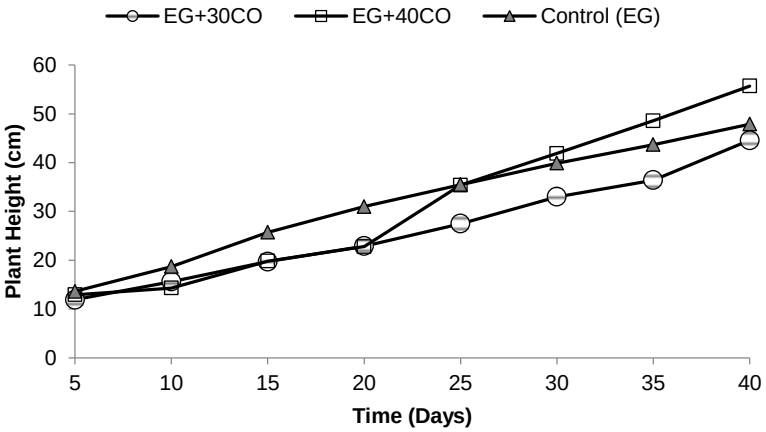

Figure 3: Impact of crude oil contamination on plant height

4.0 Conclusion

The phytoremediation capabilities of elephant grass planted in crude oil contaminated soil has been investigated. There was a gradual decrease in the total hydrocarbon content, with > 80% removal achieved within 40 days in 1 kg samples contaminated with 30 ml and 40 ml of crude oil. The pH, total viable bacterial count and total fungal count were monitored during the study. The observed increase in total viable bacterial counts and total fungal counts particularly in the treated soil samples may indicate that rhizodegradation involving associated bacteria and fungi in the root zone was a principal removal mechanism. The plants grew steadily with no evidence of inhibition due to crude oil contamination at the levels considered in this study, further confirming their suitability for environmental remediation applications.

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