

# *Pennisetum purpureum* Improved Polycyclic Aromatic Hydrocarbons Removal in Weathered-Petroleum Contaminated Soil

M. L. Riskuwa-Shehu, H. Y. Ismail, A. Y. Fardami, U. B. Ibrahim

## ABSTRACT

Petroleum hydrocarbons are among the major driving forces of advancement in the last Century. Some of the hydrocarbons especially polycyclic aromatics are however of health and environmental significance, due to their recalcitrance and persistence leading to adverse effects on health and ecosystem stability. A number of treatment technologies have been used to cleanup hydrocarbon contaminants and the use of phytoremediation technology have recently been described as promising. In this study, phytoremediation of weathered crude oil contaminated soil was carried out in a microcosm using *Pennisetum purpureum* for 60 days. Pristine soil samples were collected and mixed with weathered petroleum contaminated soil to achieve 5%, 25%, 35% and 50% w/w contamination levels. Bacterial species in the rhizosphere were isolated and identified and residual oil was extracted and analyzed using GC-MS. Results showed that there was high bacterial population in rhizosphere ( $5.0 \times 10^5$  cfu/g to  $6.4 \times 10^5$  cfu/g) than non-rhizosphere soil ( $2.4 \times 10^5$  cfu/g to  $4.0 \times 10^5$  cfu/g); and *Bacillus* spp. (64.71%) were observed to be predominant in the rhizosphere followed by *Micrococcus* spp. (17.65%), *Pseudomonas aeruginosa* (5.88%), *Klebsiella pneumoniae* (5.88%) and *Flavobacterium* sp. (5.88%). Hydrocarbon concentration in the rhizosphere was reduced by 82.5%, 60.5%, 58.0% and 48.8% respectively. Complex polycyclic aromatic hydrocarbon compounds detected in the control using GC-MS were significantly reduced or completely degraded. Polycyclic aromatic hydrocarbons such as anthracene, naphthalene, fluorene, benzo (a) anthracene, pyrene and chrysene were significantly reduced at a rate ranging between 13.33% and 97.54%. Based on the rate of PAHs reduction observed in this study, it was evident that *P. purpureum* supports cleanup of persistent hydrocarbon contaminants in soil environment. The use of this plant in large scale petroleum hydrocarbon cleanup under field conditions should be investigated.

**Keywords:** *Pennisetum purpureum*, polycyclic aromatic hydrocarbons, rhizodegradation, rhizosphere.

**Published Online:** May 17, 2022

**ISSN:** 2684-5199

**DOI :** 10.24018/ejbio.2022.3.3.285

**M. L. Riskuwa-Shehu**

Department of Microbiology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University Sokoto, Nigeria.  
(e-mail:mlriskuwa@hotmail.com)

**H. Y. Ismail\***

Department of Microbiology, Faculty of Science, University of Maiduguri, Nigeria.

(e-mail: yismailh@gmail.com)

**A. Y. Fardami**

Department of Microbiology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University Sokoto, Nigeria.

(e-mail: aminufy@gmail.com)

**U. B. Ibrahim**

Department of Microbiology, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University Sokoto, Nigeria.

(e-mail: farox@gmail.com)

*\*Corresponding Author*

## I. INTRODUCTION

Environmental contamination with crude and refined petroleum which results from increasing demands for oil products is among the global environmental issues in this century (Sojini & Ejeromedoghene, 2019). Excessive use of petroleum products have led to an intensification of environmental pollution (Pinedo *et al.*, 2013). Major components of the crude petroleum are hydrocarbons, which consist of saturated, unsaturated and polycyclic aromatics; which are considered as seriously hazardous environmental pollutants (Lundstedt *et al.*, 2007; Dudhagara *et al.*, 2016). Even with the advancement in energy technology and serious global ecosystem challenge, the world still depends significantly on petroleum for energy production, commerce, and industry; making its exploitation and transport unavoidably a continuous process. This situation exposes aquatic and terrestrial environments to constant risk of oil spills (Jin *et al.*, 2019). The major problem associated with

petroleum hydrocarbons is toxicity to plants, humans and environment (Arellano *et al.*, 2017; Yadav *et al.*, 2018). Damaging effects of contamination due to petroleum and its derived products on plant, animals, microbes and ecosystem have been documented (Ordinioha & Brisibe, 2013; Freedman, 2018).

The degree of toxicity of crude oil and its products depends on the chemical compounds present and their concentration (Environmental Protection Agency; EPA, 1999). In addition, spilled oil volume, residual oil volume on site, impacted area environment, response, recovery, and cleanup timing determines crude oil toxicity (Mohamadi *et al.*, 2016). Different biological species, including both eukaryotes and prokaryotes, and their life stages respond differently to the pollutants (Hao *et al.*, 2004). Depending on the climatic conditions, hydrodynamics and geographical location, spilled crude oil undergoes several weathering processes, like evaporation, dissolution, dispersion, sedimentation, photo-oxidation and biodegradation (Acosta-González *et al.*, 2015).

When hydrocarbon contamination prolonged for a long time, the compounds become strongly bound and only recalcitrant fraction of hydrocarbons which are not readily bioavailable prevail (Semple *et al.*, 2001). Although it may be considered less toxic than freshly contaminated environment, the effect of persistent compounds in a prolonged contaminated site is enormous (Jonker *et al.*, 2006). As various petroleum components fade in the course of weathering, polycyclic aromatic hydrocarbons (PAHs) remain entrapped in the soil matrix which pose a great health risk to humans and environment.

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds comprised of two or more condensed aromatic rings (Speight, 2006). Physically, they are described as mostly colorless, white, or pale yellow solids with varying boiling and melting points (Abdel-Shafy & Mansour, 2015). Chemically they are comprised of two or more benzene rings bonded in linear, cluster, or angular arrangements and are found in many petroleum mixtures (Arey & Atkinson, 2003). In addition, they have low vapor pressure, very low aqueous solubility, light sensitivity, heat resistance; heat conductivity, emit ability, corrosion resistance, and physiological action (Akyuz & Cabuk, 2010). PAHs containing up to six fused aromatic rings are often known as “small” PAHs, and those containing more than six aromatic rings are called “large” PAHs. The major source of PAHs is the crude petroleum however; they are predominantly introduced to the environment through natural and anthropogenic combustion processes (Speight, 2006). The release of PAHs from natural sources is limited to spontaneous forest and grassland fires and volcanic emissions. On the other hand the anthropogenic sources are diverse ranging from simple processes of incineration of wood for cooking and heating to complex industrial processes such as petroleum refining, chemical manufacturing and vehicle emissions (D’Souza *et al.*, 2015). Sediments and soils are the main sinks for all the PAHs derived from pyrogenic, petrogenic, and biological activities in the environment (Abdel-Shafy & Mansour, 2015).

In the last few decades, there is increase in soil contamination in developing countries which create a great concern to environmentalists. Particular interest have been focused on petroleum contamination due to its multiple effects as a results of the presence of heavy metals and polyaromatic hydrocarbons (Devatha *et al.*, 2019). Soil as a recipient of oil contaminants undergoes significant physicochemical changes such as particle aggregation, soil aeration, atterberg limits, permeability, pH, total organic carbon and soil minerals nutrients contents which indirectly affecting the growth and development of plants and microorganisms (Akunwumi *et al.*, 2014; Devatha *et al.*, 2019; Riazi, 2021). It has been reported that daily maximum surface temperature and hydrophobicity of petroleum contaminated soils is often higher in contaminated soil and anaerobic soil conditions are easily established (Wang *et al.*, 2013). Heavy crude oil pollution can cause complete mortality of marsh vegetation (Lin & Mendelssohn, 2012; Mohamadi *et al.*, 2016). Hence, it becomes continuously detrimental and finally, its consequences lead to deprived crop growth and soil conditions (Aislabie *et al.*, 2004).

The increasing interest for a clean and sustainable environment have made the management of contaminated

soil to be given priority in developed and developing countries with a view to improving human life and ecosystem functioning (Kuppusamy *et al.*, 2019). Presently, different cleanup technologies are been deployed to recover spilled oil, limit its spread or remove it from the environment (Maceiras, 2016; Xuezhi *et al.*, 2020). Due to the variation in environmental media and specific goal designed for a cleanup exercise, different remediation technologies have been applied in the treatment of hydrocarbon pollution and some other novel processes are still emerging (Kuppusamy *et al.*, 2017). In soil environment, bioremediation is considered an ideal technology due to its safety, eco-friendliness, non-intrusiveness and non-destructiveness (Vidali, 2001). In order to enhance the rate and effectiveness of the naturally occurring remediation process, addition of different stimulation agents have been used (Bento *et al.*, 2005; Barba *et al.*, 2021). Phytoremediation which is one form of bioremediation use plants to reduce, remove, degrade or immobilize environmental contaminants like petroleum hydrocarbons (Peer *et al.*, 2006). Phytoremediation relay on the fact that plants have extensive root systems which explore large volumes of soil and support robust microbial populations in the surrounding of the root zone which accelerate the rate of disappearance of contaminants (Farrell & Germida, 2020). To date, more than 400 plant species have been profiled for remediation of different environmental contaminants (Vangronsveld *et al.*, 2019; Yaqoob *et al.*, 2019).

*Pennisetum purpureum* (*Cenchrus purpureus* (Schumach.) synonym) is one of many plants that have been exploited in phytoremediation studies (Das *et al.*, 2017; Alikasturi *et al.*, 2020; Boonmeerati and Sampanpanish, 2021). Popularly known as elephant grass, it is a major tropical grass with high biomass yielding ability and being perennial, it can grow under a wide range of conditions (CABI, 2014). It is rhizomatous with vigorous root system and can reach up to 4-7 m in height (Heuzé *et al.*, 2020). Its versatility and extended below and above ground parts are also important characteristics for a plant with good phytoremediation potentials. Its ability to tolerate high concentration of pollutants have also been reported (Liu *et al.*, 2009; Kang *et al.*, 2015; Osman *et al.*, 2020). Although there are many studies that reported the use of *P. purpureum* in phytoremediation, majority of the studies were restricted to the treatment of inorganic pollutants. To date, only few studies have investigated the use of this plant in phytoremediation of petroleum hydrocarbon contaminated soil. As a result, the present study intended to investigate the ability of the plant to remediate more recalcitrant and more persistent PAHs in weathered contaminated soil. This is with a view to harness its versatile and invasive potentials in environmental cleanup as opposed to being weed of agronomic importance.

## II. MATERIALS AND METHODS

### A. Sample Collection and Preparation

Soil samples were collected from two different locations (contaminated and uncontaminated) in Kwalkwalawa area, Sokoto (13.1246° N, 5.1994° E), Sokoto State Nigeria.

Seeds of Elephant grass were planted before they were transplanted into the treatment pots. Soils were measured proportionately and placed into pots as describe by (Erute *et al.*, 2009). Each treatments pots contained a total of 2.5g of soil samples consisting of pristine and contaminated soil in a ratio of 50%, 35%, 25% and 5% w/w. The control soil was made up of uncontaminated soil of equal weight. The setup was replicated three times and allowed to absorb moisture for two days. Soil physicochemical properties were determined as describe by Abubakar *et al.* (2015). Parameters including nitrogen content, Organic matter content, phosphorus content, Total potassium content, PH, Electrical conductivity, Calcium, Sodium, Magnesium, Cation Exchange capacity were determined.

### B. Planting of Elephant Grass

These was done as describe by (Erute *et al.*, 2009). The 2.5g of the soil samples containing different proportion of petroleum contaminated soil was amended with 5% of organic fertilizer. The plantlets of elephant grass were transplanted into the treatment pots, at two weeks growth period. The plant growth was monitored for a period of nine (9) weeks in the contaminated soil for removal of petroleum hydrocarbons; after which the rhizosphere soil was taken for microbial analysis. Residual hydrocarbon fractions in the soil were also extracted and analyzed using GC-MS.

### C. Microbiological Analysis

Rhizosphere soil samples were serially diluted and inoculated using pour plate techniques. Aliquots (1ml) of six-fold dilutions were inoculated in to nutrient agar (NA; Oxoid) and mineral salt agar (MSA; composed of (per litre at pH 7.4): 1.2 g  $\text{KH}_2\text{PO}_4$ , 1.8 g  $\text{K}_2\text{HPO}_4$ , 4.0 g  $\text{NH}_4\text{Cl}$ , 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g  $\text{NaCl}$ , 0.01 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 20 g agar and 0.01% v/v crude oil) petri plates. The plates were incubated for 24hrs and 120hrs respectively after which enumeration of total viable and hydrocarbon utilizing bacteria was conducted respectively and expressed as CFU/g of soil. Colonies growing on MSA were sub-cultured on NA to obtain pure cultures and subsequently characterized and identified based on their cultural, morphological and biochemical characteristics in accordance with determinative schemes of Barrow and Feltham (1993) and Benson (2002).

### D. Determination of Total Petroleum Hydrocarbons

#### 1) Extraction of residual oil

Ten gram (10 g) each of soil samples were weighed into Whatman extraction thimbles (that had been pre-extracted with n-hexane). The samples were extracted using the soxhlet extractor with 250 mL DCM for 16 h. The extracts were further reduced to 10 mL using a rotary evaporator and transferred into 4 mL amber vials and kept in the refrigerator for analysis.

#### 2) GC-MS analysis

Residual oils were analyzed as describe by Dhivya *et al.*, (2014) using GC-MS (Agilent Technologies 6890N Network

GC System and Agilent Technologies 5973 Network Mass selective Detector coupled with 7683B Series Injector). The model number of the column used was Agilent 122-5533 capillary column with specification: DB-5ms, 0.25 mm×30 m ×1  $\mu\text{m}$ . The carrier gas used was Helium at a flow rate of 1.2ml/min with an injection volume of 1ml. The inlet temperature was maintained at 230 °C. The oven temperature was initially at 50 °C and increased to 300 °C at a rate of 10 °C ending with 25 minutes. This temperature was held for 15minutes with a total run time of 45 minutes. The ionization mode used was electron ionization mode at 70 eV. Total Ion Count (TIC) was used for compound identification and quantitation. The spectrum of separate compounds were compared with database of the spectrum of known compounds saved in the NIST02 Reference Spectra library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software.

### E. Statistical Analysis

Data obtained from the experiments were analyzed using descriptive statistics with the aid of Graph Pad Prism 9 statistical package.

## III. RESULTS AND DISCUSSION

The physicochemical properties of the soil mixture is presented in Table I. The contaminated soil was acidic with 0.76% and 0.067% carbon and nitrogen. The Ca, Mg, K, Na and CEC contents (mol/Kg) of the soil were 0.95, 0.25, 0.97, 0.52 and  $10^6$  respectively. The soil was sandy, with low nutrients and mineral compositions. The physical properties were in the normal prevailing soil conditions obtainable in the area. Based on its physicochemical properties, the soil is said to be marginal, porous and highly aerated. Therefore, the soil conditions are suitable for the growth of *P. purpureum* since previous studies have shown that, the plant is able to survive on a broad range of soils ranging from waterlogged clay soils to excessively porous sandy soils, under a wide pH range (Rahman *et al.*, 2008). Osman *et al.* (2020) have described *P. purpureum* as indifferent to various soil nutrients and minerals concentration. Its growth rate is rarely affected by presence of inorganic pollutants like copper, lead and chromium. Its biomass generation does not strictly depends on nutrients and mineral supplementations (Islam *et al.*, 2017). These with some other qualities made the plant desirable for phytoremediation.

Bacterial population of the rhizosphere and non-rhizosphere soil was estimated and presented in Table II. Total heterotrophic and hydrocarbon utilizing bacterial populations were higher in the rhizosphere, in which uncontaminated (RS0) and contaminated (RS35) soil had the highest THB population.

TABLE I: SOIL PHYSICOCHEMICAL PROPERTIES

pH	OC %	N (%)	P (mg/Kg)	Ca (mol/Kg)	Mg (mol/Kg)	K (mol/Kg)	Na (mol/Kg)	C.E.C (mol/Kg)
5.6±0.8	0.76±0.3	0.067±0.2	0.83±0.1	0.95±0.5	0.25±0.1	0.97±0.1	0.52±0.5	10.0 ±1.2

OC: Organic Carbon, N: Nitrogen, P: Phosphorus, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, CEC: Cation exchange capacity



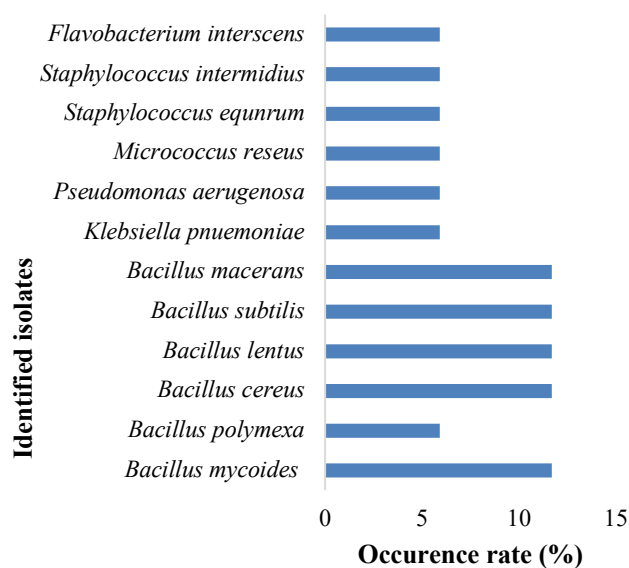
TABLE II: BACTERIAL COUNTS OF RHIZOSPHERE OF *P. PURPUREUM*

Samples	Bacterial count ( $\times 10^5$ cfu/g)	
	Total Heterotrophic Bacteria (THB)	Hydrocarbon Utilizing Bacteria (HUB)
NR0	4.00	2.00
NR5	2.40	2.80
NR25	3.20	2.20
NR35	4.90	2.80
NR50	4.00	3.20
RS0	64.0	24.0
RS5	53.0	48.0
RS25	55.0	36.0
RS35	64.0	44.0
RS50	50.0	24.0

Higher HUB were recorded in RS5 and RS35 with  $4.8 \times 10^5$  cfu/g and  $4.4 \times 10^5$  cfu/g respectively. In the non-rhizosphere soil, NR35 (THB) and NR50 (HUB) had higher bacterial counts whereas NR5 (THB) and NR0 (HUB) had the lowest counts respectively. Higher bacterial counts in the rhizosphere is a good indication of the plant's role in improving microbial population primarily through root exudates secretion. Plants are known to release from their roots compounds that are readily utilized by microorganisms as carbon and energy sources. Zahui *et al.*, (2021) reported the bacterial population in the rhizosphere of *P. purpureum* to reach  $7.7 \times 10^6$  cfu/g in a constructed wetland and attributed it to exudates secreted by plants in the root zone. There are indication that high number of PAH degraders also correlates well with the amount of PAH biodegradation activity in soil and soils with a high total PAH content contain more PAH utilizers ( $10^5$ - $10^{10}$  bacteria per gram of soil) than soils with low PAH content (Cerniglia, 1993). Studies have shown that plant species, as well as ecological habitats, have a substantial influence on the structure of rhizosphere-associated microbial populations (Guo *et al.*, 2019). It has been estimated that bacterial populations in the rhizosphere, are 10–100 times higher than in bulk soil which results to positive rhizosphere effect (Pinton *et al.*, 2007; Olahan *et al.*, 2016). Presence of rhizosphere have been linked to increased microbial biomass in soil which is an important early indicator of improvement in soil quality and degradation of contaminants (Gichangi *et al.*, 2016).

A total of 17 bacterial species were isolated and identified from the rhizosphere soil as hydrocarbon utilizing species (Figure 1). *Bacillus mycoides*, *Bacillus cereus*, *Bacillus lentus*, *Bacillus subtilis* and *Bacillus macerans* were the predominant species with 11.7% rate of occurrence each. *Staphylococcus intermedius*, *Flavobacterium interescens*, *Staphylococcus equirum*, *Micrococcus reus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus polymexa* were least prevalent with 5.9% occurrence rate each. Occurrence of these bacterial species in rhizosphere of several plant species have severally been reported. *Pseudomonas* spp. and *Bacillus* spp. belong to the largest groups of rhizosphere bacteria (Brimecombe *et al.*, 2007). Most of these species have been reported as potent hydrocarbon degrading bacteria in different environments (Margesin *et al.*, 2003; Xu *et al.*, 2018). Their ability to utilize hydrocarbons as sole energy and carbon source highlight their bioremediation capabilities. The bacterial species earlier reported as PAHs degraders are known to produce dioxygenase enzymes which are necessary for ring cleavage (Gupta *et al.*, 2019). From this results, it is evident that the

plant's rhizosphere harbors a diverse bacterial species which is important for plant growth and environmental cleanup. Bacteria associated with plant roots are often responsible for plant growth promotion. Previous culture-independent molecular studies have revealed a diverse bacterial species capable of nitrogen fixation in the *P. purpureum* rhizosphere of which majority of the species were then unidentified (Videira *et al.*, 2013). Plant growth promotion is critical to proper growth of plants used in phytoremediation. Rhizospheric bacteria help plants through their metabolic detoxification mechanisms which convert toxic compounds that are detrimental to plant growth into non-toxic substrate (Adieze *et al.*, 2012). Additionally, procurement of nutrients for plant assimilation and controlling the proliferation of phytopathogens are major activities of bacteria within plant rhizosphere (Gilick, 2012). The bacterial diversity observed in this study is desirable considering the fact that no single microbial species is capable of completely degrading petroleum hydrocarbon contaminants but entire microbial community (Varjani, 2017). Bacteria are the most active petroleum degrading microbes as they are primary degraders of a wide range of target constituents of the petroleum hydrocarbons. They are the most abundant and diverse especially in contaminated site (Chikere & Ekwuabu, 2014). Most of bacterial species reported so far for their PAHs degradation ability are members of *Proteobacteria*, *Actinobacteria*, and *Firmicutes* (Cerniglia, 1993; Peng *et al.*, 2008; Gabriele *et al.*, 2021).

Fig. 1. Occurrence rate of hydrocarbon utilizing bacteria in the rhizosphere of *P. purpureum*.

Analysis of residual oil extract showed a significant reduction in concentration of various PAH compounds (Table III). Low molecular weight PAHs were more degraded than the higher molecular PAHs with the exception of pyrene and Benzo (a) anthracene in some of the treatments. In RS5 naphthalene was reduced by 92.28% and other PAHs in this treatment were reduced by > 70% with Benzo (a) anthracene and Chrysene been degraded at lower rate. Highest degradation rate was observed in treatments with lesser contaminants (RS5 and RS25) where degradation of individual compounds was > 50% with the exception of Pyrene which was reduced by only 30% - a rate that was

contrary to that of RS35 where 97.92% degradation rate was observed. In RS50 however, low reduction rate was generally observed with < 50% reduction in PAHs concentration. Despite the recalcitrance of PAHs, the use of *P. purpureum* and associated rhizobacteria as phytoremediators have caused significant reduction of its concentration especially where the contamination is at lower level. In all the treatments, naphthalene degradation was prominent possibly due to its

lower molecular weight. *In-vitro* biodegradation studies have shown that naphthalene can be entirely degraded by bacteria even when a single bacterium was used in the culture broth (AL Sbani *et al.*, 2020). Studies have shown that PAHs with four and five fused benzene rings are more resistant to biodegradation than PAHs with two or three rings (Cerniglia, 1993).

TABLE III: PERCENTAGE REMOVAL OF PAHS IN THE RHIZOSPHERE OF *P. PURPUREUM*

Compound	No. of Rings	Degradation rate (%)			
		RS5	RS25	RS35	RS50
Naphthalene	2	92.28	95.54	37.93	23.53
Fluorene	3	77.00	91.71	23.08	43.75
Phenanthrene	3	80.00	88.00	37.50	13.33
Anthracene	3	88.00	55.00	36.36	16.67
Pyrene	4	77.14	30.00	97.92	18.75
Benzo(a)anthracene	4	73.33	83.33	75.00	18.75
Chrysene	4	65.00	33.33	95.45	20.00

nd: Not detected

A number of bacterial species such as *Pseudomonas* sp., *Alcaligenes* sp., *Staphylococcus* sp. and *Arthrobacter* sp. have been reported to completely degrade or co-metabolize higher molecular weight PAHs, such as fluoranthene, pyrene, fluorene and benz[a]anthracene albeit at slower rate (Monna *et al.*, 1993). The degradation rate observed in this study was earlier reported in some phytoremediation exercises. In our previous study using *Cajanus cajan* and *Lablab purpureus*, naphthalene was completely degraded in both cases, whereas pyrene, fluorene and fluoranthene were either completely degraded or significantly reduced (Riskuwa-Shehu & Ismail, 2018). Degradation of PAHs in plant rhizosphere does not necessarily follow a regular pattern when it is exposed to different concentration of contaminants as observed in this study. Our findings are supported by the work of Moradi *et al.* (2021) who observed different rate of acenaphthene ( $74.63 \pm 0.78$  in control), fluoranthene ( $71.18 \pm 0.56$  in 2.5%), and anthracene ( $69.45 \pm 6.33$ ,  $55.66 \pm 4.38$  and  $35.97 \pm 0.22$  in 5.0, 7.5 and 10% contamination respectively) degradation in the rhizosphere of *Avicennia marina*.

The success of PAHs removal observed in this study could be attributed to plant-microbe synergism. Recent studies have shown that rhizospheric microorganisms significantly relay on root exudates, since most of the exudates are readily available sources of nutrients. As a result, microbial species become chemotactically attracted, which results to increased biomass and contaminants degradation (Hoang *et al.*, 2021; Mishra *et al.*, 2021). The microbes in return, support plant growth by minimizing contaminants' toxicity, promoting root elongation, enhance uptake and stabilization due to secretion of chelators; and degradation (Vangronsveld *et al.*, 2019; Ismail *et al.*, 2021). Although the interaction is plant specific, the rate of PAHs degradation was reported to increase in rhizosphere of different plant species compared to bulk soil as observed by Li *et al.* (2021) using *Echinacea purpurea* and *Festuca* sp. The work of Gabriele *et al.* (2021) have shown that removal of pyrene from soil was better achieved by plant-bacteria synergism than plant-fungi interaction or phytoextraction. In addition to bacterial degradation, there are speculations that extracellular enzymes secreted by plant in the rhizosphere could play an important role for the degradation of PAHs like pyrene (Agarwal *et al.*, 2020). Furthermore, the weathered soil might have over time

allowed the evolution of competent bacterial species capable of high rate of PAHs degradation and their activity was significantly promoted upon planting of *P. purpureum*. In a study involving pyrene degradation using *Potamogeton crispus*, plant growth resulted in more enhanced bioavailability (+73.9%) and biodegradation activity (+277%) of pyrene in aged sediments as compared to freshly contaminated sediments where only 13.1% bioavailability and 150% biodegradation rate was observed during 36 days period (Meng & Chi, 2017).

#### IV. CONCLUSION

Removal of PAHs from weathered hydrocarbon-contaminated soil was monitored for 9 weeks using *P. purpureum*. Enrichment in rhizosphere bacterial population was generally observed and diverse bacterial species were isolated and identified with predominance of *Bacillus* spp. High rate of PAHs degradation was observed in soil with low contamination rate as opposed to high contamination where lowest degradation rate was observed. Naphthalene, fluorene, phenanthrene and anthracene were degraded by > 50% in soil contaminated with  $\leq 25\%$  contaminants whereas pyrene, benzo (a) anthracene and chrysene were degraded more in soil with 35% contamination. Despite the desirable qualities of *P. purpureum* for phytoremediation, less attention have so far been given to its application in hydrocarbon cleanup especially PAHs. The present work would therefore provide an insight into its use for large scale PAHs phytoremediation.

#### CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

#### REFERENCES

- Abdel-Shafy, H. I. & Mansour, M. S. M. (2015). A Review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation, *Egyptian Journal of Petroleum*.
- Abubakar, B. M., Lawal, A., & Argungu, L. A., (2015). Preliminary evolution of soil from Kwakwalawa (Sokoto) and Bakolori (Talata Mafara) regions used for rice and farming activities, *Journal of*

- Agricultural Science and Technology*, 5: 247-251.
- Acosta-González, A., Martirani-von Abercron, S.-M., Rosselló-Móra, R., Wittich, R.-M. & Marqués, S. (2015). The effect of oil spills on the bacterial diversity and catabolic function in coastal sediments: A case study on the prestige oil spill. *Environ. Sci. Pollut. Res.* 22, 15200–15214.
- Adieze, I. E., Orji, J. C., Nwabueze, R. N. & Onyeze, G. O. C. (2012). Hydrocarbon stress response of four tropical plants in weathered crude oil contaminated soil in microcosms. *Int. J. Environ. Stud.* 69(3):490–500.
- Agarwal, P., Giri, B. S. & Rani, R. (2020). Unravelling the role of rhizospheric plant-microbe synergy in phytoremediation: a genomic perspective. *Curr. Genom.*
- Aislabie, J. M., Balks, M. R. & Foght, J. M. (2004). Hydrocarbon spills on Antarctic soils: Effects and management. *Environmental Science & Technology*, 38(5): 1265–1274. doi: 10.1021/es0305149
- Akunwumi, I. I., Diwa, D. & Obianigwe, N. (2014). Effects of crude oil contamination on the index properties, strength and permeability of lateritic clay. *Int J Appl Sci Eng Res* 3:816–824.
- Akyuz, H. M. & Cabuk, H. (2010). Gas-particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. *Science of the Total Environment*; 408: 5550–5558.
- AL Sbani, N. H., Abdullah, S. R. S., Idris, M., Hasan, H. A., Halmi, M. I. E., Jehawi, O. H., & Ismail, N. 'Izzati. (2020). PAH-degrading rhizobacteria of *Lepironia articulata* for phytoremediation enhancement. *Journal of Water Process Engineering*, 101688.
- Alikasturi, A. S., Mokhtar, M. I., Zainuddin, M. A., Serit, M. E., & Rahim, N. S. A. (2020). Phytoremediation of lead in mineral, distilled and surface water using *Pennisetum purpureum* and *Allium fistulosum*. *Materials Today: Proceedings*, 31, A175-A179.
- Arellano, P., Tansey, K., Balzter, H. & Tellkamp, M. (2017). Plant family-specific impacts of petroleum pollution on biodiversity and leaf chlorophyll content in the Amazon rainforest of Ecuador. *PLoS ONE*, 12(1): e0169867.
- Arey, J. & Atkinson, R. (2003). Photochemical reactions of PAH in the atmosphere, In: Douben, P. E. T. (Ed.), PAHs: *An Ecotoxicological Perspective* (pp. 47–63) John Wiley and Sons Ltd, New York.
- Barba, S., Villasenor, J., Rodrigo, M. A., & Cañizares, P. (2021). Biostimulation versus bioaugmentation for the electro-bioremediation of 2, 4-dichlorophenoxyacetic acid polluted soils. *Journal of Environmental Management*, 277, 111424.
- Barrow, G. & Feltham, K. (1993). Cowan and Steel's manual for identification of medical bacteria. London: Cambridge University Press.
- Benson, H. (2002). Microbiological applications laboratory manual. 8th ed. New York: McGraw-Hill Companies.
- Bento, F. M., Camargo, F. A., Okeke, B. C., & Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresour. technology*, 96(9), 1049-1055.
- Boonmeerati, U., & Sampanpanish, P. (2021). Enhancing Arsenic phytoextraction of dwarf napier grass (*pennisetum purpureum* cv. mott) from gold mine tailings by electrokinetics remediation with phosphate and EDTA. *Journal of Hazardous, Toxic, and Radioactive Waste*, 25(4), 04021027.
- Brimecombe, M. J., De Leij, F. A. M. & Lynch, J. M. (2007). Rhizodeposition and microbial populations. In: R. Pinton., Z. Varanini. and P. Nannipieri. (eds). *The Rhizosphere: biochemistry and organic substances at the soil-plant interface*, (pp 73–109). New York, CRC Press, Taylor & Francis Group.
- CABI, 2014. *Invasive Species Compendium*. Wallingford, UK: CAB International
- Cerniglia, C. E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. *Current Opinion in Biotechnology*, 4(3), 331–338.
- Chikere, C. B., & Ekwuabu, C. B. (2014). Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. *African Journal of Environmental Science and Technology*, 8(6), 401-406.
- D'Souza, R., Varun, M., Lakhani, A., Singla, V. & Paul, M. S. (2015). PAH contamination of urban soils and phytoremediation. In: A.A. Ansari (eds.), *Phytoremediation: Management of Environmental Contaminants*, 1.
- Das, A., Belgaonkar, P., Raman, A. S., Banu, S., & Osborne, J. W. (2017). Bioremoval of lead using *Pennisetum purpureum* augmented with *Enterobacter cloacae*-VITPASJ1: A pot culture approach. *Environmental Science and Pollution Research*, 24(18), 15444-15453.
- Devatha, C. P., Vishnu Vishal, A., & Purna Chandra Rao, J. (2019). Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. *Applied Water Science*, 9(4).
- Dhivya, H., Seeli, B., Madhan, and Akila, S. (2014). Production of amphiphilic surfactant molecule from *Saccharomyces cerevisia* MTCC 181 and its Protagonistin Nanovesicle Synthesis. *International Journal of Science, Invention*; 3(11):16-23.
- Dudhagara, D. R., Rajpara, R. K., Bhatt, J. K., Gosai, H. B., Sachaniya, B. K. & Dave, B. P. (2016). Distribution, sources and ecological risk assessment of PAHs in historically contaminated surface sediments at Bhavnagar coast, Gujarat, India. *Environmental pollution*. 213: 338-46.
- Environmental Protection Agency EPA (1999). *Understanding Oil Spills And Oil Spill Response*. EPA Office of Emergency and Remedial Response. OSWER 9200.5-104A.
- Erute, M. O., Mary, Z. & Gloria, O. (2009). Effect of crude oil on growth of the weed (*Paspalum scrobiculatum* L.) phytoremediation potential of plant. *African Journal of Environmental Science and Technology*, 3 (9), 229-233.
- Farrell, R. E., & Germida, J. J. (2002). Phytotechnologies: Plant-based Systems for remediation of oil impacted soils. Available from <https://www.esaa.org/wp-content/uploads/2015/06/02-09FarrellPaper>
- Freedman, B. (2018). Environmental science. Canada: Dalhousie University Libraries Digital Editions.
- Gabriele, I., Race, M., Papirio, S., & Esposito, G. (2021). Phytoremediation of pyrene-contaminated soils: A critical review of the key factors affecting the fate of pyrene. *Journal of Environmental Management*, 293, 112805.
- Gichangi, E. M., Njarui, D. M. G., Gatheru, M., Ndungu-Magiroy, K. W., & Ghimire, S. R. (2016). Effects of *Brachiaria* grass cultivars on soil microbial biomass carbon, nitrogen and phosphorus in soils of the semi-arid Eastern Kenya. *Climate Smart Brachiaria Grasses for Improving Livestock Production in East Africa-Kenya Experience*, 179.
- Glick, B. R. (2012) Plant growth promoting bacteria: mechanisms and applications. *Scientifica*.
- Guo, D., Fan, Z., Lu, S., Ma, Y., Nie, X., Tong, F., & Peng, X. (2019). Changes in rhizosphere bacterial communities during remediation of heavy metal-accumulating plants around the Xikuangshan mine in southern China. *Scientific reports*, 9(1), 1-11.
- Gupta, G., Kumar, V., & Pal, A. K. (2019). Microbial degradation of high molecular weight polycyclic aromatic hydrocarbons with emphasis on pyrene. polycyclic aromatic compounds.
- Heuzé, V., Tran G., Giger-Reverdin S. & Lebas F., (2020). Elephant grass (*Pennisetum purpureum*). Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. <https://feedipedia.org/node/395>
- Hoang, S. A., Lamba, D., Seshadria, B., Sarkarb, B., Choppalaa, G., Kirkhamc, M. B., & Bolana, N. S. (2021). Rhizoremediation as a green technology for the remediation of petroleum hydrocarbon contaminated soils. *Journal of Hazardous Materials*, 401, 123282.
- Islam, M. S., Sarker, N. R., Habib, M. A., Ali, M. Y. & Yeasmin, T. (2017). Effect of different soil types on growth and production of Napier-4 at the Regional Station of BLRI. *Asian Journal of Medical and Biological Research* 3(2): 182-185.
- Jin, M., Shi, W., Yuen, K. F., Xiao, Y., & Li, K. X. (2019). Oil tanker risks on the marine environment: An empirical study and policy implications. *Marine Policy*, 108, 103655.
- Jonker, M. T., Brils, J. M., Sinke, A. J., Murk, A. J. & Koelmans, A. A. (2006). Weathering and toxicity of marine sediments contaminated with oils and polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* 25, 1345-1353.
- Kang, D. J., Seo, Y. J., Saito, T., Suzuki, H. & Ishii, Y. 2012. Uptake and translocation of cesium-133 in napier grass (*Pennisetum purpureum* Schum.) under hydroponic conditions. *Ecotoxicology and environmental safety* 82: 122-126.
- Kuppusamy, S., Maddela, N. R., Megharaj, M., & Venkateswarlu, K. (2019). Case Studies on Remediation of Sites Contaminated with Total Petroleum Hydrocarbons. *Total Petroleum Hydrocarbons*, 225–256.
- Li, N., Liu, R., Chen, J., Wang, J., Hou, L., & Zhou, Y. (2021). Enhanced phytoremediation of PAHs and cadmium contaminated soils by a *Mycobacterium*. *Science of the Total Environment*, 754, 141198.
- Lin, Q. & Mendelssohn, I. A., (2012). Impacts and recovery of the deep water horizon oil spill on vegetative structure and function of coastal salt marsh in the Northern Gulf of Mexico. *Environmental Science & Technology*, 46(7): 3737–3743.
- Liu, X., Shen, Y., Lou, L., Ding, C. & Cai, Q. (2009). Copper tolerance of the biomass crops Elephant grass (*Pennisetum purpureum* Schumach), Vetiver grass (*Vetiveria zizanioides*) and the upland reed (*Phragmites australis*) in soil culture. *Biotechnology Advances* 27(5):633-640.
- Lundstedt, S., Paul, A. W., Christine, L. L., Krista, D. L., Iain, B. L., Lars, Ö., Peter, H. & Mats, T. (2007). Sources, fate, and toxic hazards of oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites. *AMBIO: A Journal of the Human Environment* 36, no. 6 (2007): 475-485.



- Maceiras, R. (2016). Emerging technologies for soil remediation of hydrocarbons. *Pharm Anal Chem*, 2(01), 102.
- Margesin, R., Labbé, D., Schinner, F., Greer, C. W., & Whyte, L. G. (2003). Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. *Appl. Environ. Microbiol.* 69, 3085–3092.
- Meng, F. & Chi, J. (2017). Effect of *Potamogeton crispus* L. on bioavailability and biodegradation activity of pyrene in aged and unaged sediments. *J. Hazard Mater.*
- Mishra, S., Mulla, S. I., Saha, S., Kharat, A S., More, N. & Bharagava, R. N. (2021). Involvement of synergistic interactions between plant and rhizospheric microbes for the removal of toxic/hazardous contaminants. In: Sharma, A. (Ed.). *Microbes and Signaling Biomolecules against Plant Stress*. Rhizosphere Biology.
- Mohamadi, B., Liu, F. & Xie, Z. (2016). Oil spill influence on vegetation in nigeria and its determinants. *Pol. J. Environ. Stud.* 25(6): 2533-2540.
- Monna, L., OioPa, T. & Kodama, T. (1993). Microbial degradation of dibenzofuran, fluorene, and dibenzo-p-dioxin by *Staphylococcus auriculans* DBF63. *Appl Environ Microbiol* 1993, 59:285-289.
- Moradi, B., Maivan, H. Z., Hashtroudi, M. S., Sorahinobar, M., & Rohloff, J. (2021). Physiological responses and phytoremediation capability of *Avicennia marina* to oil contamination. *Acta Physiologiae Plantarum*, 43(2), 1-12.
- Sojino, O. S., & Ejeromedoghene, O. (2019). Environmental challenges associated with processing of heavy crude oils. Processing of heavy crude oils - challenges and opportunities.
- Olahan, G. S., Sule, I. O., Garuba, T. & Salawu, Y. A. (2016). Rhizosphere and non-rhizosphere soil mycoflora of *Cochorus olitorius* (Jute). *Science World Journal*, 11 (No 3):23-26.
- Ordinioha, B. & Brisibe, S. (2013). The human health implications of crude oil spills in the Niger Delta, Nigeria: An interpretation of published studies. *Nigerian Medical Journal*; 54:10-16.
- Osmana, N. A., Roslana, A. M., Ibrahima, M. F., & Hassana, M. A. (2020). Potential use of *Pennisetum purpureum* for phytoremediation and bioenergy production: A mini review. *Sci. Rep.*, 10, 6613.
- Peer, W. A., Baxter, I. R., Richards, E. L., Freeman, J. L., & Murphy, A. S. (2006). Phytoremediation and hyperaccumulator plants. In *Molecular Biology of Metal Homeostasis and Detoxification* (pp. 299–340). Springer.
- Pinedo, J., Ibáñez, R. J., Lijzen, P. A. & Irabien, A. (2013). Assessment of soil pollution based on total petroleum hydrocarbons and individual oil substances. *Journal of Environmental Management*, 130, 72-79.
- Pinton, R., Varanini, Z. & Nannipieri, P. (2007). The rhizosphere: biochemistry and organic substances at the soil-plant interface. *CRC Press*: Boca Raton, FL, USA.
- Peng, R. H., A. S. Xiong, Y. Xue, X.Y. Fu, F. Gao, W. Zhao, Y. S. Tian, Q. H. Yao. (2008). Microbial biodegradation of polyaromatic hydrocarbons, *FEMS Microbiol. Rev.* 32, 927–955,
- Rahman, M. M., Ishii, Y., Niimi, M. & Kawamura, O. (2008). Effect of salinity stress on dry matter yield and oxalate content in napier grass (*Pennisetum purpureum* Schumach). *Asian-Australasian Journal of Animal Sciences*, 21(11), 1599-1603.
- Riazi, M. R. (2021). Oil spill occurrence, simulation, and behavior (1st ed.). CRC Press.
- Riskuwa-Shehu, M. L., & Ismail, H. Y. (2018). Isolation of endophytic bacteria and phytoremediation of soil contaminated with polycyclic aromatic hydrocarbons using *Cajanus cajan* and *Lablab purpureus*. *Bioremediation Science and Technology Research*, 6(1):26-30. <https://journal.hibiscuspublisher.com/index.php/BSTR/issue/view/42>.
- Semple, K. T., Doick, K. J., Wick, L. Y. & Harms, H. (2007). Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. *Environ. Pollut*, 150, 166-176.
- Speight, J. G. (2006). *The Chemistry and Technology of Petroleum*. CRC Press.
- Vangronsveld, J., Weyens, N., Thijs, S., Dubin, D., Clemmens, M., Van Geert, K., et al. (2019). Phytoremediation – Code of good practice. *Ovam*, 1-132.
- Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresour. Technol.* 223, 277–286.
- Vidali, M. (2001). Bioremediation: An overview. *Pure and applied Chemistry*, 73(7), 1163-1172.
- Videira, S. S., e Silva, M. D. C. P., de Souza Galisa, P., Dias, A. C. F., Nissinen, R., Divan, V. L. B. et al. (2013). Culture-independent molecular approaches reveal a mostly unknown high diversity of active nitrogen-fixing bacteria associated with *Pennisetum purpureum*—a bioenergy crop. *Plant and Soil*, 373(1), 737-754.
- Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang X. & Wang, G. (2013). Effects of crude oil contamination on soil physical and chemical properties in Momoge Wetland of China. *Chinese Geographical Science*, 23(6), 708–715.
- Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., et al. (2018). Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. *Front. Microbiol*, 9, 2885.
- Xuezhi, D., Anum, A. A., Ishaq, M., Tariq, S., & Qudratullah, K. (2020). Remediation methods of crude oil contaminated soil. *World J Agri & Soil Sci.*, 4(3), 34–46.
- Yadav, I. C., Devi, N. L., Li, J., & Zhang, G. (2018). Polycyclic aromatic hydrocarbons in house dust and surface soil in major urban regions of Nepal: Implication on source apportionment and toxicological effect. *Science of the Total Environment*, 616-617, 223–235.
- Yaqoob, A., Nasim, F. H., Sumreen, A., Munawar, N., Zia, M. A., Choudhary, M. S. et al. (2019). Current scenario of phytoremediation: progresses and limitations. *International Journal of Biosciences*, 14(3), 191-206.
- Zahui, F., Yeo, É., Ouattara, J. & Coulibaly, L. (2021). Bacteriological quality of a forage grass (*Pennisetum purpureum* Schumach) used in constructed wetland removing domestic wastewater pathogenic microorganism. *Journal of Environmental Protection*, 12, 311-327.