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Axonopus compressus: A Resilient Phytoremediatior of Waste Engine Oil Contaminated Soil

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

A phytoremediation study using *Axonopus compressus* (carpet grass) was carried out on three levels of simulated waste engine oil (WEO) contamination of soil for a period of 12 months.
The microorganisms associated with the rhizosphere region of the plant were the bacteria *Bacillus* sp. and fungi *Aspergillus niger* and *Aspergillus carbonarius.*
The plants were harvested at 1, 3, 6, 9 and 12 months intervals and the respective fresh weights were taken at the respective harvest times. The plants remained resilient despite the level of WEO contamination and results of some physicochemical parameters of the contaminated soils measured showed that the plant was able to phytoremediate the WEO contamination.

Keywords: Phytoremediation; Axonopus compressus; waste engine oil; contamination; soil.

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1. INTRODUCTION

Following increased use of automobiles, millions of gallons of waste engine oil (WEO) are disposed in trash, on land or into sewers, with the potential for contaminating soil, groundwater and surface water [1]. Such has resulted in diverse problems of environmental pollution which has assumed an unprecedented proportion in many parts of the world [2,3].

One of the world's most common environmental problems is soil contamination by petroleum hydrocarbons [4]. This is because total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants [5]. Generally, the accumulation of contaminants in soils can have destructive effects on the environment and human health. Contaminants present in soils can enter the food chain and seriously affect animal and human health [6]. Therefore, suitable solutions for the removal or control of these soil contaminants must be found.

Various physical, chemical and biological methods are suitable for decontaminating relatively small areas while they are expensive to use over large areas such as the ones contaminated by industrial substances, oil products and mining sites [7,8].

A number of innovative physical and chemical technologies are available to remediate soil contaminated with hydrocarbon pollutants. For example, soil washing, vapor extraction, encapsulation and solidification/ stabilization have been successful [9]. These methods, however, are expensive, and may only be partly effective. In addition, public pressures may restrict the field utilization of such intensive techniques. Recent studies indicate that plant roots provide a beneficial habitat for hydrocarbon-degrading microbes. Therefore, Phytoremediation, which is the use of vegetation i.e. plants to extract, sequester, or detoxify pollutants is a better remediation method and is environmentally friendly and visually attractive, and the structure of the soil is highly maintained [4,10,11]. It is a technology that has lasted almost two decades [12,13,14].

The aim of this study therefore is to assess the phytoremediation potential of *Axonopus compressus* on three levels of simulated waste engine oil contaminated soil.

2. MATERIALS AND METHODS

2.1 Simulation of WEO Contamination

Waste Engine Oil (WEO) was pooled in a 50 litres gallon from the Total Station (Pit Section) at Anloga Junction in Kumasi, Ghana. Top soil was excavated at a depth of 15cm from the Botanic Garden of the Kwame Nkrumah University of Science and Technology packed in sac bags and moved to the Department of Theoretical and Applied Biology Plant House.

Three (3) levels of waste engine oil contamination of soil were simulated in the following soil-oil mixture ratios:

	1%	**5%**	**10%**
	50 g Oil 5 kg Soil	250 g Oil 5 kg Soil	500 g Oil 5 kg Soil
Where Therefore,	100 g Oil = 135 ml, 50 g Oil = 67.5 ml 250 g Oil = 337.5 ml and 500 g oil = 675 ml.		

These were designated samples HCSSL1, HCSSL2 and HCSSL3.

The height of the buckets used was 20 cm each while the level of soil in each bucket was 16 cm.

The formula used for the above mixtures is as described in [15].

All the mixtures were done in four replicates.

2.2 Planting Material

Tufts of *Axonopus compressus* were acquired from a commercial horticulturist in Owerri, Imo State, Nigeria, packed in a Cellophene bag, got cleared by the National Agricultural Quarantine Service (NAQS), Murtala Mohammed International Airport, Lagos, Nigeria and were flown to Ghana for this research. These tufts were transplanted into a wooden box (length 50cm, width 60cm and height 20cm) for multiplication and subsequently planted unto the diverse soil/WEO mixes.

Carpet grass *(Axonopus compressus)* is a grass which is often used as a permanent pasture, ground cover and turf in moist, low fertility soils particularly in shaded situations. It belongs to the Kingdom – Plantae; Order – Poales; Family – Poaceae; Genus – Axonopus; Species – compressus.

2.3 Measurement of Fresh Weight of Plant

The *Axonopus compressus* plants were harvested five (5) times during the 12-month study period (i.e. months 1, 3, 6, 9 and 12). At each harvest, the fresh weights of the plants were taken using a digital scale and data recorded.

2.4 Collection of Rhizosphere Soil

The plant was carefully uprooted at harvest times, shaken carefully for 2 minutes to remove attached large soil particles. Rhizosphere soils were collected as those that were stuck to the roots of the plant within 3 mm of thickness from the immediate root periphery. The soils were pooled together and taken to lab for microbiological analyses [16].

2.5 Culture of Rhizosphere Soil / Characterization and Identification of the Rhizosphere Microorganisms

The rhizosphere soils were cultured according to the methods outlined in [17]. The pure cultures were further characterised and identified at the CABI Microbial Services Laboratory, Bakelam lane, Egbam, Surrey, United Kingdom.

Characterization and identification of fungal isolates was done using ITS rDNA sequencing analysis. Sequencing reactions were undertaken by comparing the sequence obtained with those available in the European Molecular Biology Laboratory (EMBL) through the European Bioinformatics Institute (EBI) and the International Subcommission on *Trichoderma* and *Hypocrea* (ISTH).

For the bacterial isolate, molecular assay was carried out on the sample using nucleic acid as a template. Following DNA extraction, Polymerase Chain reaction (PCR) (TP 63) was employed to amplify copies of the partial 16S fragment of rDNA in vitro. Sequencing reactions (TP 66) were undertaken and identification was done by comparing the sequence with those available in EMBL via the EBI.

2.6 Monitoring the Phytoremediation of the WEO Contaminated Soil

2.6.1 Monitoring total heterotrophic and hydrocarbon utilizing microbial counts

This was carried out five (5) times during the 12-month study period, namely at 1 month, 3 months, 6 months and 12 months, for the respective soil samples (HCSSL1, HCSSL2 and HCSSL3) by the monitoring of the heterotrophic and hydrocarbon utilizing bacterial and fungal counts using the appropriate media and methods already mentioned [17].

2.6.2 Analysis of selected physico-chemical parameters

The respective soil samples were examined for some physico-chemical parameters like pH (using distilled water method); total hydrocarbon content (using APHA colorimetry method); total organic carbon (using refluxing method); phosphate (P+) content (using colorimetry method); nitrate (N+) content (using brucine method) and sulphate (S+) content (using turbidimetric method).

Polycyclic aromatic hydrocarbon (PAH) content of the respective samples was carried out using the cold extraction GC-FID method while a check on the heavy metals content was done using the AAS:APHA methods 3010-3110 [18,19,20].

2.7 Statistical Analysis

All data from this study were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 20) by analysis of variance on ranks to compare the means of the different treatments.

3. RESULTS

The mean weight of *Axonopus compressus* during the harvest times is as shown in Table 1. It ranged from 12.75 ± 0.50g to 109.75 ± 5.44g in all the treatments plants for all the samples. The weights of *A. compressus* increased as the treatment period (months) progressed for sample HCSSL3; in samples HCSSL1 and HCSSL2, the weight of the plants increased at 1 month after treatment (MAT) and decreased by subsequent harvest times till 6 MAT and 9 MAT respectively. The respective weights varied significantly ($p<0.05$).

The Rhizospere organisms isolated from the plant is the Bacteria, *Bacillus* sp and Fungi, *Aspergillus niger & Aspergillus carbonarius.* These are shown in Table 2.

The Total Heterotrophic Bacterial and Fungal Counts (THBC and THFC) in Simulated Soil Samples cultivated with *Axonopus compressus* is shown in Table 3.

Table 1. Mean weight of *Axonopus compressus* cultivated in simulated WEO contaminated soils over a period of 12 months

Sample	Month	M±SD
	0	37.50 ± 3.11^{f}
	1	38.75 ± 3.40^{e}
HCSSL1	3	36.75 ± 2.75^{e}
	6	12.75 ± 0.50^{a}
	9	26.75 ± 0.50^{c}
	12	30.00 ± 0.81^{cd}
	0	37.75 ± 3.40^{e}
	1	38.75 ± 3.40^{e}
HCSSL2	3	34.75 ± 2.75^{de}
	6	17.00 ± 0.82^{ab}
	9	13.25 ± 0.50^{a}
	12	20.50 ± 3.87^{b}
	0	37.25 ± 3.10^{e}
HCSSL3	1	38.75 ± 3.40^{e}
	3	47.50 ± 2.64^{f}
	6	102.75 ± 0.96^{g}
	9	109.75 ± 5.44^{h}
	12	107.50 ± 2.38^{g}

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)
M ± SD is Mean plus or minus standard deviation
HCSSL1 – Hydrocarbon contaminated simulated soil level 1; Hydrocarbon contaminated simulated soil level 2; Hydrocarbon contaminated simulated soil level 3.

From the results, the contaminated soils had decreasing THBC at all levels of contamination with a range of values in decreasing order of 3.51 ± 0.05 to 3.95 ± 0.01; 0.00 ± 0.00 to 3.95 ± 0.02 and 0.00 ± 0.00 to 3.91 ± 0.02 cfu/g for samples HCSSL1, HCSSL2 and HCSSL3 respectively. The total heterotrophic fungal counts (THFC) for samples HCSSL1 and HCSSL2 had a rise in counts by 1 month after treatment (MAT) respectively while sample HCSSL3 had decreasing counts from 1 MAT till 12 MAT with a range of values from 3.35 ± 0.06 to 4.10 ± 0.04; 0.00 ± 0.00 to 4.05 ± 0.05 and 0.00 ± 0.00 to 3.57 ± 0.03 cfu/g for samples HCSSL1, HCSSL2 and HCSSL3 respectively.

The Hydrocarbon Utilizing Bacterial and Fungal Counts (HUBC & HUFC) in Simulated Soil Samples cultivated with *Axonopus compressus* is shown in Table 4.

The hydrocarbon utilizing bacterial counts from the *A. compressus* treatment increased till 3 MAT for all levels of contamination followed by a decline from 6 MAT to the 12th month after treatment.

The hydrocarbon utilizing fungal counts in the set ups over the 12 months study period shows that in sample HCSSL1, the count was 4.05 ± 0.04 at month 0, this increased to 4.21 ± 0.06 by 1 MAT, followed by a decrease from month 3 to 12.

Sample HCSSL2 soils had their hydrocarbon utilizing fungal counts increase till 3 MAT, followed by a consistent decrease till 12 MAT.

Samples HCSSL3 soils had their hydrocarbon utilizing fungal counts also increase consistently from 0 MAC (month after contamination) to 3 MAT, followed by reduced counts. The respective counts were significantly different between the levels.

The Physicochemical parameters of simulated hydrocarbon contaminated soil Samples cultivated with *Axonopus compressus* is shown in Table 5.

The pH values in sample HCSSL1 ranged from 5.23 ± 0.02 to 5.60 ± 0.02; in sample HCSSL2, it was from 5.33 ± 0.01 to 5.78 ± 0.02 while the range in sample HCSSL3 was from 5.40 ± 0.02 to 5.45 ± 0.02.

The Total Hydrocarbon Content (THC) of sample HCSSL1 range from 96.00 ± 0.03 to 1800.00 ± 0.03; HCSSL2 from 4600.00 ± 0.02 to 10140.00 ± 0.05 and sample HCSSL3 from 13100.00 ± 0.02 to 21320.00 ± 0.03, all in reverse order.

All the values for THC differ significantly at $p<0.05$.

The percentage Total Organic Carbon (TOC) contents of the respective samples range from 5.41 ± 0.04 to 6.69 ± 0.04 for sample HCSSL1; 6.21 ± 0.03 to 10.22 ± 0.02 for sample HCSSL2 and 10.02 ± 0.02 to 11.61 ± 0.04 for sample HCSSL3 in reverse order.

All values at the respective levels differ significantly ($p<0.05$).

The nitrate contents of all the samples increased over the study period. In samples HCSSL1, HCSSL2 and HCSSL3, it had a range from 1.76 ± 0.05 to 25.38 ± 0.05; 26.76 ± 0.03 to 61.66 ± 0.04 and 35.02 ± 0.05 to 64.03 ± 0.03 mg/kg respectively. The values have differed significantly at $p<0.05$ at the times of harvest.

The phosphate content of the respective samples ranged from 20.58 ± 0.06 to 26.25 ± 0.06 for sample HCSSL1; 204.75 ± 0.04 to 217.40 ± 0.05 for sample HCSSL2 and 205.25 ± 0.06 to 246.90 ± 0.04 mg/kg for sample HCSSL3. The phosphate values differ significantly at $p<0.05$.

Table 2. Identification of microorganisms isolated from the rhizosphere region of the phytoremediation plant

Plant type	Species	Shape	Chromogenesis	Elevation	Margin	Appearance	Identity
Axonopus compressus	Bacteria	Circular & Undulating, motile, tree-like and spreading	Creamy White	Raised	Smooth and Glistening	Translucent	*Bacillus subtilis*
	Fungi	Circular	Yellow with scanty black spores				*Aspergillus niger*
	Fungi	Circular	White and fluffy with dark spores				*Aspergillus carbonarius*

Table 3. Total Heterotrophic bacterial and fungal counts in simulated soil samples cultivated with *A. compressus* (mean log cfu/g)

Month	THBC			THFC		
	HCSSL1	HCSSL2	HCSSL3	HCSSL1	HCSSL2	HCSSL3
0	3.95 ± 0.01^{e}	3.95 ± 0.02^{e}	3.91 ± 0.02^{e}	3.35 ± 0.06^{a}	3.53 ± 0.07^{c}	3.57 ± 0.03^{e}
1	3.83 ± 0.02^{d}	3.57 ± 0.05^{d}	3.69 ± 0.01^{d}	4.10 ± 0.04^{e}	4.05 ± 0.05^{d}	3.52 ± 0.06^{e}
3	3.76 ± 0.05^{c}	3.51 ± 0.01^{c}	3.63 ± 0.02^{c}	3.82 ± 0.05^{d}	3.59 ± 0.06^{c}	3.40 ± 0.07^{d}
6	3.74 ± 0.03^{c}	3.35 ± 0.05^{a}	3.51 ± 0.03^{b}	3.71 ± 0.02^{c}	3.25 ± 0.03^{b}	3.27 ± 0.04^{c}
9	3.67 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.52 ± 0.06^{b}	0.00 ± 0.00^{a}	3.17 ± 0.03^{b}
12	3.51 ± 0.05^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.40 ± 0.04^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)
Mean cfu/g – mean logarithm of colony forming units per gram of soil sample

Table 4. Hydrocarbon utilizing bacterial and fungal counts in simulated soil samples cultivated with *Axonopus compressus* (mean log cfu/g)

Time (Month)	HUBC			HUFC		
	HCSSL1	HCSSL2	HCSSL3	HCSSL1	HCSSL2	HCSSL3
0	4.24 ± 0.05^{b}	4.32 ± 0.05^{bc}	4.38 ± 0.02^{b}	4.05 ± 0.04^{cd}	3.92 ± 0.05^{b}	3.80 ± 0.06^{b}
1	4.33 ± 0.01^{c}	4.37 ± 0.05^{c}	4.49 ± 0.02^{c}	4.21 ± 0.06^{e}	4.15 ± 0.07^{c}	4.04 ± 0.02^{c}
3	4.39 ± 0.01^{d}	4.47 ± 0.02^{d}	4.57 ± 0.02^{d}	4.13 ± 0.06^{de}	4.18 ± 0.07^{c}	4.14 ± 0.02^{d}
6	4.32 ± 0.03^{c}	4.38 ± 0.05^{c}	4.50 ± 0.01^{c}	3.97 ± 0.04^{c}	4.11 ± 0.05^{c}	3.96 ± 0.06^{c}
9	4.19 ± 0.01^{b}	4.26 ± 0.02^{b}	4.41 ± 0.02^{b}	3.75 ± 0.06^{b}	3.94 ± 0.07^{b}	3.83 ± 0.03^{b}
12	4.11 ± 0.03^{a}	4.16 ± 0.05^{a}	4.31 ± 0.01^{a}	3.60 ± 0.04^{a}	3.66 ± 0.05^{a}	3.65 ± 0.06^{a}

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)
Mean log cfu/g – mean logarithm of colony forming units per gram of soil sample
HUBC – Hydrocarbon utilizing bacterial count; HUFC – Hydrocarbon utilizing fungal count; HCSSL1 – Hydrocarbon contaminated simulated soil level 1; Hydrocarbon contaminated simulated soil level 2; Hydrocarbon contaminated simulated soil level 3.

Table 5. Physicochemical parameters of simulated WEO contaminated soil samples cultivated with *Axonopus compressus*

Samples	Time	Parameters					
		pH	THC (mg/kg)	TOC (mg/kg)	N^+ (mg/kg)	P^+ (mg/kg)	S^+ (mg/kg)
HCSSL1	0 month	5.23 ± 0.02^{a}	1800.00 ± 0.03^{l}	6.69 ± 0.04^{l}	1.76 ± 0.05^{a}	20.58 ± 0.06^{a}	80.25 ± 0.07^{a}
	1 month	5.26 ± 0.03^{a}	1710.00 ± 0.04^{e}	6.20 ± 0.05^{e}	4.27 ± 0.06^{b}	22.73 ± 0.07^{b}	162.50 ± 0.03^{b}
	3 months	5.28 ± 0.02^{ab}	1520.00 ± 0.03^{d}	6.02 ± 0.04^{d}	9.86 ± 0.05^{c}	23.50 ± 0.06^{c}	290.75 ± 0.07^{e}
	6 months	5.43 ± 0.02^{ef}	1200.00 ± 0.02^{c}	5.93 ± 0.03^{c}	17.10 ± 0.04^{d}	24.03 ± 0.05^{d}	474.65 ± 0.06^{g}
	9 months	5.53 ± 0.02^{g}	1010.00 ± 0.03^{b}	5.71 ± 0.04^{b}	22.24 ± 0.05^{e}	25.53 ± 0.06^{e}	941.53 ± 0.07^{m}
	12 months	5.60 ± 0.02^{h}	960.00 ± 0.03^{a}	5.41 ± 0.04^{a}	25.38 ± 0.05^{f}	26.25 ± 0.06^{f}	998.65 ± 0.07^{o}
HCSSL2	0 month	5.33 ± 0.01^{bc}	10140.00 ± 0.05^{l}	10.22 ± 0.02^{k}	26.76 ± 0.03^{g}	204.75 ± 0.04^{g}	285.25 ± 0.05^{c}
	1 month	5.35 ± 0.02^{cd}	10040.00 ± 0.02^{k}	10.02 ± 0.02^{j}	36.20 ± 0.03^{i}	206.60 ± 0.04^{i}	287.50 ± 0.05^{d}
	3 months	5.45 ± 0.04^{ef}	8965.00 ± 0.05^{j}	9.75 ± 0.06^{i}	41.21 ± 0.07^{k}	210.50 ± 0.03^{k}	300.75 ± 0.05^{f}
	6 months	5.63 ± 0.01^{h}	6300.00 ± 0.05^{i}	9.32 ± 0.02^{h}	47.17 ± 0.03^{m}	212.30 ± 0.04^{l}	494.63 ± 0.05^{h}
	9 months	5.65 ± 0.02^{h}	5120.00 ± 0.02^{h}	8.65 ± 0.02^{g}	56.12 ± 0.03^{o}	214.90 ± 0.04^{m}	981.40 ± 0.05^{n}
	12 months	5.78 ± 0.02^{i}	4600.00 ± 0.02^{g}	6.21 ± 0.03^{e}	61.66 ± 0.04^{q}	217.40 ± 0.05^{n}	1002.50 ± 0.06^{p}
HCSSL3	0 month	5.48 ± 0.02^{fg}	21320.00 ± 0.03^{r}	11.61 ± 0.04^{p}	35.02 ± 0.05^{h}	205.25 ± 0.06^{h}	774.75 ± 0.07^{l}
	1 month	5.45 ± 0.02^{ef}	21280.00 ± 0.03^{q}	11.43 ± 0.04^{o}	40.48 ± 0.05^{j}	209.60 ± 0.06^{j}	781.75 ± 0.07^{j}
	3 months	5.43 ± 0.04^{ef}	18050.00 ± 0.05^{p}	11.12 ± 0.06^{n}	43.51 ± 0.07^{l}	219.60 ± 0.03^{o}	793.50 ± 0.05^{k}
	6 months	5.43 ± 0.02^{ef}	16040.00 ± 0.03^{o}	10.82 ± 0.04^{m}	51.06 ± 0.05^{n}	260.55 ± 0.06^{r}	851.50 ± 0.07^{l}
	9 months	5.45 ± 0.02^{ef}	15000.00 ± 0.03^{n}	10.66 ± 0.04^{l}	57.83 ± 0.05^{p}	233.65 ± 0.06^{p}	1066.55 ± 0.07^{q}
	12 months	5.40 ± 0.02^{de}	13100.00 ± 0.02^{m}	10.02 ± 0.02^{j}	64.03 ± 0.03^{r}	246.90 ± 0.04^{q}	1081.75 ± 0.05^{r}

Means ± SD (in same column) with different letters in superscripts differ significantly ($p < 0.05$)

HCSSL1 – Hydrocarbon contaminated simulated soil level 1; Hydrocarbon contaminated simulated soil level 2; Hydrocarbon contaminated simulated soil level 3.

The sulphate content of the respective samples range from 80.25 ± 0.07 to 998.65 ± 0.07 for sample HCSSL1; 285.25 ± 0.05 to 1002.50 ± 0.06 for sample HCSSL2 and 774.75 ± 0.07 to 1081.75 ± 0.05 mg/kg for sample HCSSL3. The sulphate contents of the respective samples are significantly different at $p<0.05$.

4. DISCUSSION

4.1 Mean Weight of *Axonopus compressus* Cultivated in Simulated Hydrocarbon Contaminated Soils over a Period of 12 Months

The reduced weight of the plants at the harvest times is attributed to the stress imposed on the plants by the waste engine oil contaminant. Hydrocarbon utilizing bacteria and fungi in soil compete with the plants for oxygen and mineral nutrients. This competition results in oxygen exhaustion which can create an anaerobic condition. When such condition is thus created, it results in a microbial generation of phytotoxic compounds, for example hydrogen sulphide. Furthermore, the physical structure of the soil is affected by the contaminant, this results in a decrease in its capacity to store moisture or air [21].

4.2 Rhizosphere Microorganisms Associated with *Axonopus compressus* Cultivated in Simulated Hydrocarbon Contaminated Soils

The bacterial isolate from the rhizosphere region of the plant is *Bacillus subtilis.* Microorganisms have been used in conjunction with plants to remediate contaminated soils. These microorganisms are plant growth promoting rhizo-bacteria or fungi found usually in the rhizosphere region of plants. Such organisms are known to stimulate plant growth through diverse mechanisms e.g. by supply of nutrients, production of phytochromes [22], production of chelating agents [23], nitrogen fixation and specific enzyme activity [6].

Bacillus species are well known rhizosphere residents of many plants and usually show plant growth promoting activities [24]. *B. subtilis* also known as “grass bacillus” is a gram positive bacterium commonly found in soil and has the ability to tolerate extreme environments because of its protective endospore [25].

To corroborate our finding, [26] had isolated *B. subtilis* from the rhizosphere of *Axonopus compressus* in their study on the “rhizoremediation of polyaromatic hydrocarbon content of a model waste diesel engine oil polluted soil by some local lawn plant species in Benin city, Nigeria”.

The fungi *Aspergillus niger* and *Aspergillus carbonarius* were isolated from the rhizosphere of *Axonopus compressus.* Species of *Aspergillus* are reputed to be very effective in removal of hydrocarbons and heavy metals in soil [27,28,29]. More specifically, researchers have reduced heavy metal contents in contaminated soils using *A. niger* [30,31,32,33,34].

4.3 Total Heterotrophic Bacterial and Fungal Counts in Simulated Soil Samples Treated with *Axonopus compressus*

The total heterotrophic bacterial counts in the soil treated with *Axonopus compressus* had declining counts as the phytoremediation treatment was ongoing at all three levels of contamination. This suggests that the soil in the *A. compressus* treatment had unfavourable soil moisture conditions during early plant establishment. *A. compressus* has a fibrous root system [35]; this creates a need for water around the root region. Due to the hydrophobic nature of petroleum contaminated soil, there is water percolation through the soil column without sufficiently wetting the soil in the root zone. As a result, the plant suffered drought which led to an increase in the plant hormones production of abscisic acid and ethylene [36]. These acid and ethylene is responsible for the induction of defence reactions that lead to an accumulation of antimicrobial phytoalexins [37] which had an additional inhibitory effect on soil bacteria.

4.4 Hydrocarbon Utilizing Bacterial and Fungal Counts in Simulated Soil Samples Treated with *Axonopus compressus*

The hydrocarbon utilizing bacterial and fungal counts of the soils at all levels during phytoremediation treatment maintained a normal microbial growth curve. This suggests that there was a synergy between the bacteria and fungi degrader groups in the course of the treatment [38], facilitating the degradation of the waste engine oil contaminant.

4.5 Physicochemical Parameters of Simulated Hydrocarbon Contaminated Soil Samples Treated with *Axonopus compressus*

The pH values of the soils during the phytoremediation treatment were within the acidic range. The acidic pH can be attributed to the production of organic acids due to the metabolism of the rhizobacteria and fungi of the plant. [39] reported that *Bacillus* and *Aspergillus* species secrete organic acids and lower the pH in their vicinity; this further explains why the samples had an acidic pH range as *Bacillus subtilis* was isolated from the rhizosphere region of the plant and the fungi *Aspergillus niger* and *carbonarius* were isolated.

The total hydrocarbon contents of the soils treated with *A. compresus* reduced by 47%, 55% and 39% respectively for samples HCSSL1, HCSSL2 and HCSSL3. This implies that the plant used for the phytoremediation treatment has the capacity to extract the hydrocarbon content in the soils thus making it available for other organisms and reducing environmental hazards associated with the contamination [10]. The mechanism responsible for this hydrocarbon contents removal is the growth and activity of hydrocarbon degrading microorganisms in the rhizosphere of the plant [40].

Reduction of total hydrocarbon content of the soils was evident in the *A. compressus* treatment. This corroborates with the report of [41] who report that plants of the grass family (Poacea) are particularly suitable for phytoremediation because of their multiple ramified root systems.

The total organic carbon contents of the respective soils were high at the start of the phytoremediation treatment. This was as a result of the introduction of large amounts of hydrocarbons into the soils. However, the treatment of these contaminated soils was effective, resulting in the reduction of the total organic carbon contents of the respective samples by 19%, 39% and 14%. When large amounts of hydrocarbon is added to soil, the carbon tends to stimulate bacteria and fungi rapidly which attack the carbon. The synergy between the rhizosphere bacteria and fungi within the rhizosphere region of the plant and the plant's exudates thus facilitated a breakdown of the organic carbon contents of the respective soils resulting in the reductions.

The treatment plant restored the soil macronutrients as the phytoremediation study progressed. [42] reports that phytoremediation is effective in the restoration of depleted soil nutrients which may have been caused by the introduction of organic or inorganic contaminants. Increase in phosphate and sulphate may be as a result of nodulation by the roots of the plants. The increases in nitrate and phosphate contents may also be because of the increasing sulphate content. [43] reported correlations of sulphate with other soil properties.

5. CONCLUSION

The implication of the findings from this study is that *Axonopus compressus* has the tenacity to withstand the deleterious effects of waste engine oil contamination and the capacity to phytoremediate hydrocarbon concentration in soil effectively in any geographical region of the world.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adeyemi AO. Bioaccummulation of arsenic by fungi. Am. J. Environ. Sci. 2009;5:364-370.
2. Ahmad I, Ansari I, Aqil F. Biosorption of Ni, Cr and Cd by metal tolerant *Aspergillus niger* and *Penicillium* sp. using single and multi-metal solution. Indian J. Exp. Biol. 2005a;44:73-76.
3. Amro MM. Factors affecting chemical remdiation of oil contaminated water-wetted soil. Chemical Engineering & Technology. 2004;27(8):890-894.
4. April W, Sims RC. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere. 1990;20:253-256.
5. APHA. Standard method for the examination of water and waste water.

American Public Health Association, Washington DC. 1985;256.

6. ASTM. Methodology for the comparison of petroleum oil by gas chromatography. American Sosiety for Testing and Materials, Method D. 1982;3328-3378.
7. Bank MK, Mallede H,Rathbone H. Rhizosphere microbial characterization in petroleum contaminated soil. Soil and Sediment Contamination. 2003;12(3):371-385.
8. Boller T, Mattoo AK, Suttle JC. The plant Hormone Ethylene, CRC Press, Boca Raton; 1991.
9. Calvo P, Ormeno-Orrillo E, Martinez-Romero E, Znniga D. Characterization of *Baeillus* isolates of potato rhizoshere from Andean soils of Peru and their potential PGPR characteristics. Brazilian Journal of Microbiology. 2010;41(4).
10. Chekol T, Vough LR, Chaney RL. Phytoremediation of polychlorinated biphenyl-contaminated soils: The rhizosphere effect. Environment International. 2004;30:799-804.
11. Chikere BO, Chijioke-Osuji CC. Microbial diversity & physico-chemical properties of a crude oil polluted soil. Nigerian Journal of Microbiology. 2006;*20*(2):1039-1046.
12. Cole MA, Liu Z. Plant and microbial establishment on pesticide-contaminated soil amended with compost. Bioremediation through rhizosphere technology. Journal Environmental Microbiology. 1994;21:16-22.
13. Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. Hazard. Mater. 2007;146:270-277.
14. Cunningham SD, Anderson TA, Schwab PA, Hsu FC. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 1996;56:55-114.
15. Dejong E. The effect of a crude oil spill on cereals. Environ. Pollut. Ser. A. 1980;22: 187-196.
16. Edwin-Wosu NL. Phytoremediation (Series 5): Organic carbon, matter, phosphorus and nitrogen trajectories as indices of assessment in a macrophytic treatment of hydrocarbon degraded soil environmental Biology. Pelagia Research Library, European Journal of Experimental Biology. 2013;3(3):11-17.
17. ERI. Analysis of Extractible Total Petroleum Hydrocarbons (ETPH), using Methylen Chloride (DCM) Gas Chromatography/ Flame Ionization Detecion. Environmental Research Institute, University of Connecticut; 1999.
18. Escalante-Espinosa E, Gallegos-Martinez ME, Favela-Torres E, Gutlerrez-Rojas M. Improvement of the hydrocarbon phytoremediation rate of *Cyperus laxus* Lam. Inoculated with a microbial consortium in a model system. Chemosphere. 2005;59:405-413.
19. Frick CM, Farrell RE, Germida JJ. Assessment of phytoremediation as an in situ technique for cleaning oil contaminated sites. Petroleum Technology Alliance, Canads: Calgary, Canada. A. 1999;88.
20. Glick BR. Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. Biotechnology Advances 2003;21:239-244.
21. Glizk BR, Karaturovic DM, Newell PC. A novel procedure for rapid isolation of plant growth promoting pseudomonads. Canadian Journal of Microbiology. 1995;41(6):533-536.
22. Huang XD, El-Alawi Y, Gurska J, Glick BR, Greenberg BM. A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem. J. 2005;81:139-147.
23. Ikhajiagbe B, Anoliefo GO. Natural attenuation of a 14-month-old waste engine oil polluted soil. Journal of Soil Science and Environmental Management. 2011;2(7):184-192.
24. Ikhajiagbe B, Chijioke-Osuji CC. Heavy Metal content and microbial composition of the rhizosphere of *Eleusine indica* within an auto-mechanic workshop In Benin City, Nigeria. Journal of the Ghana Science Association. 2012;14(2).
25. Ikhajiagbe B, Anoliefo GO, Imoni A. Rhizoremediation of polycyclic hydrocarbon content of a model waste diesel engine oil-polluted soil by some local lawn plant species in Benin city, Nigeria. Studia Universitatis Babes-Bolyai Biologia. LX. 2015;2:5-16.
26. Indiana Department of Environmental Management (IDEM). Environmental Facts. Indiapolis, IN: Office of pollution prevention and technical assistance; 2002. Available:www.state.in.us/idem/oppta/recycling/education/facts.pdf

27. Joshi PK, Swamp A, Maheshwari S, Kumar R, Singh N. Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J. Microbiol. 2011;51:482-487.
28. Kamney AA, Vanderlelie D. Chemical and biological parameters as fools to evaluate and improve heavy metal phytoremediation. Bioscience Reports. 2000;20(4):239-258.
29. Khan AG. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J. Trace Elem. Med. Biol. 2005;18:355-364.
30. Khan AG, Kuek C, Chaudry TM, Khoo CS, Hayes WJ. Role of plants mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere. 2000;41:197-207.
31. Kustreba L. Oil spill remediation efforts in the Middle East. Dept. of horticultural Science, Univ. of Minnesota, St. Paul; 1999.
32. Larcher W. Physiological Plant Ecology. Springer, Berlin. Madigan, M. and Martinko J. (eds) (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall; 1995.
33. Massoumi A, Cornfield AH. Total Sulphur and water soluble sulphate contents of soils and their relation to other soil properties. Journal of the Science of Food and Agriculture. 1964;15(9):623-625.
34. Pal TK, Bhattacharyya S, Basumajumdar A. Cellular distribution of bioaccummulated toxic heavy metals in *Aspergillus niger* and *Rhizopus arrhizus*. Int. J. Pharma. Bio. Sci. 2010;1:1-6.
35. Ramasamy RK, Lee JT, Cho JY. Toxic cadmium ions removal by isolated fungal strain from e-waste recycling facility. J. Environ. Appl. Biores. 2012;1:1-4.
36. Raskin I, Kumar PBAN, Dushenkov S, Salt DE. Bioconcentration of heavy metals by plants. Current Opininons in Biotechnology. 1994;5:285-290.
37. Salt DE, Blaylock M, Rumor NP, Dushenkuv V, Ensley BD, Het I, Raskin I. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnol. 1995;13:468-474.
38. Salt DE, Smith RD, Raskin I. Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology. 1998;49:643-668.
39. Swamy AVVS. Biofertilizers for environmental protection. Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences, 2014;1, S1.
40. Thippeswamy B, Shiva-Kumar CK, Krishnappa M. Accummulation potency of heavy metals by *Saccharomyces* sp. indigenus to paper mill effluent. J. Environ. Res. Dev. 2012b;6:439-445.
41. United States Environmental Protection Agency. Solid Waste and Emergency Response, EPA 542-R-92-011. Number 45 Oct. 2000. United States Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory; 2000.
42. Zafar S, Aquil F, Ahmad J. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Biores. Techol. 2007;98: 2557-2561.

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